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ONCOLOGY OVERVIEW

SELECTED ABSTRACTS

ORIGINAL

ON

RECEPTOR AND BINDING PROTEINS

FEBRUARY 27, 1979

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service • National Institutes of Health •
National Cancer Institute

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INTRODUCTION

This ONCOLOGY OVERVIEW on receptors and binding proteins related to cancer contains abstracts from 1965 to the 1977 entries in the CANCERLINE data base. The strategy used for the on-line data base search is available on request from the CIDAC office and can be entered at any MEDLARS/MEDLINE terminal system in order to obtain continuing updates in this area.

The scope of this OVERVIEW includes articles on cell surface receptors, DNA and RNA binding proteins, carcinogen binding proteins, steroid hormone receptors, cyclic nucleotide binding proteins, vitamin-related binding proteins, drug binding proteins and proteins binding other components which affect cellular functions.

This OVERVIEW was prepared by Gerald Litwack of the Fels Research Institute and Department of Biochemistry, Temple University School of Medicine and Barbara E. Hindman of the CIDAC for Virology, Immunology and Biology, The Franklin Institute Research Laboratories.

EDITORIAL COMMENTARY

The early observations which provided a basis for the study of cytoplasmic steroid hormone receptors began appearing in the early and middle 1960s. Considerable research up to the present day supports the idea that the actions of steroid hormones at the cellular level are mediated by specific binding proteins, or receptors. In the case of steroid hormone action, transcriptional processes are affected by the steroid-receptor complex which is translocated to the nucleus from the cytoplasm.

A large number of recent studies have indicated the usefulness of quantifying steroid-hormone receptors, especially the estrogen receptor, in biopsy specimens of breast cancer. The levels of this receptor have been used in predicting the potential effectiveness of further endocrine intervention to control the growth of such tumors.

Another line of investigation established that polypeptide hormones interact with receptors located in cellular membranes of target cells. These receptors have higher affinities for the more potent active hormones, lower affinities for the less potent hormones and are saturable. In other respects these cell membrane receptors resemble steroid hormone receptors, in that they seem to be required for mediation of hormone action at the cellular level. Recently, investigators have begun to consider whether hormones which bind to cell membrane receptors might be internally translocated and have further actions within the cell, perhaps in the nucleus.

Cyclic nucleotide binding proteins are often involved in the secondary effects of hormones binding to cell membrane receptors. The activity of such a complex may involve an adenylate cyclase (or similar enzyme) whose catalytic site is exposed to the interior soluble cytoplasm. The hormone-receptor complex at the outer side of the cell membrane generates a conformational change in the cyclic nucleotide synthesizing enzyme in the interior and causes fluctuations in the cytoplasmic content of cyclic nucleotides. Elevations, for example, result in increased binding to cyclic nucleotide binding proteins which frequently appear to be regulatory subunits of inactive protein kinases. Attachment of the cyclic nucleotide to its binding protein results in the dissociation of the complex from the inactive protein kinase, releasing the active catalytic subunit and initiating a chain of events culminating in the alterations associated with the cellular response to the particular hormone originally bound at the outer cell membrane.

The resemblance of certain aromatic hydrocarbon carcinogens to the fundamental planar ring structure of steroid hormones led to study of the question whether chemical carcinogens, which have been shown to interact with DNA, may be facilitated in this action by a specific receptor. Clear evidence in this direction has been obtained for certain inducers of the microsomal cytochrome P-450 system and it is of interest that some of these inducers are carcinogenic. Although recent efforts have focused attention to the interaction of carcinogen metabolites directly with the DNA of the genome, there is increasing interest in the possibility that specific carcinogen receptor proteins may intervene between the microsomal metabolism of the potentially toxic agent and the interaction of the metabolites with the cell nucleus.

In recent years, knowledge has dramatically increased concerning specific cell membrane receptors and binding sites for agents, other than hormones, which regulate cellular functions, such as lectins, mitogens, viruses, carbohydrates and glycoproteins. Investigation of the "receptors" will aid in understanding cell growth and cell transformation. In addition, nucleic acid binding proteins appear as hallmarks of viral transformation within mammalian cells.

These different species of receptors and binding proteins seem to relate in various ways to initiation of the neoplastic process, to cell transformation, and to growth of cancer cells. The large number of abstracts in this collection can serve to introduce the reader to recent literature on receptors of various kinds and their roles in normal and cancer cells as well as in the process of cellular transformation and carcinogenesis.

I. STEROID HORMONE RECEPTORS

A. The Estrogen Receptor in Human Breast and Prostate Cancer and Predictive Value for Endocrine Therapy

1. STEROIDS AND HUMAN BREAST CANCER.

McGuire WL, Chamness GC, Costlow ME, Richert NJ
Univ. Texas Health Science Center, San Antonio, Tex. 78284

J Steroid Biochem; 6(5):723-728 1975

Studies of specific estrogen receptors and of the mechanisms of action of the receptor-estrogen complex in human mammary tumors, induced rat mammary tumors, and other estrogen target tissues are reviewed. Estrogen has been shown to act directly on the normal mammary gland to promote growth and differentiation, and to also stimulate the release of pituitary prolactin . However, experiments showing prolactin stimulation of tumor growth in the absence of ovarian steroids, albeit of brief duration, have suggested only a secondary role of estrogen. Studies of the nuclear binding of estrogen receptors have demonstrated the presence of estrogen receptors in the cytoplasm of dimethylbenz(a)anthracene - induced hormone-dependent rat mammary tumors, and the accumulation of injected estrogen in their cell nuclei in vivo, apparently still bound to a 4-5S form of the receptor. Experiments employing the R323OAC transplantable mammary carcinoma have indicated that the chromatin of the autonomous breast tumor cells possesses the capacity for extensive receptor-estrogen interaction. Cytoplasmic receptor-estrogen also binds equally well to nuclei prepared from target and nontarget tissues, in a manner strictly proportional to the available receptor-estrogen. The quantitation of estrogen receptor in human breast cancer specimens is described. Receptors in 154 primary tumors and 72 metastatic tumors have been assayed. The estrogen receptor values in primary tumors range from zero to almost 1,000 femtoM/mg of cytosol protein. Data from 436 treatment trials in 380 patients are also reviewed, and clinical correlations are presented. Thirty-three percent of 211 ablative therapy treatment trials yielded objective tumor regression; 8% of 94 trials in patients with negative tumor receptor values were successful, while 55% of 107 trials in patients with positive tumor receptor values succeeded. Of 170 additive therapy trials, 8% of 82 trials in patients with negative tumor receptor values succeeded, compared to 60% of 85 trials in patients with positive tumor receptor values. In addition, 27% of 55 trials yielded responses to a variety of endocrine therapies, including antiestrogens and aminoglutethimide . Thus the data reveal that estrogen receptor assays can be helpful in predicting the results of endocrine therapy for metastatic breast cancer. (32 refs)

2. OESTROGEN RECEPTORS IN BREAST CANCER: A CHANGING CONCEPT.

Lecercq G, Heuson JC, Beboel MC, Mattheiem WH
No affiliation given

Br Med J; 1(5951):185-189 1975

Estrogen receptors in 214 primary breast cancers and 168 metastatic deposits were assayed by measuring the binding affinity of their cytosol fraction for 3H-estradiol-17 beta at 18 C. Receptors were found in 156 (73%) of the primary tumors and in 98 (58%) of the metastatic tumors. When the findings were considered chronologically, these proportions reached 82% and 70%, respectively, in the second half of the study as against 64% and 46% in the first half. Receptors were not found in samples of normal breast tissue, but small amounts were present in tissue from some hyperplastic lesions and in male gynecomastia. Receptor concentrations in the malignant samples were evenly distributed over a wide range of values, suggesting that even "negative" tumors might contain trace amounts undetectable by the method used. Each tumor was characterized by a given level of receptor concentration. In most cases, the amounts found in the invaded axillary nodes and their corresponding primary tumors were the same. Quantitative measurement of receptor content might assess the degree of hormone dependence of a tumor. It is therefore suggested that quantitative rather than qualitative assessment should provide an appropriate criterion for studies of biochemical and clinical correlations.

3. THE ROLE OF ESTROGEN RECEPTORS IN PREDICTING SUBSEQUENT THERAPY FOR RECURRENT BREAST CANCER.

Takeuchi O, Di Veccia L, Bronn DG, Pace WG, Minton JP
Dept. Surgery, Ohio State Univ. Coll. Medicine, Columbus, 43210

Ohio State Med J; 72(3):153-157 1976

Estrogen receptors (ER) were assayed using the dextran-charcoal method in 20 recurrent breast cancers and compared with values in 23 benign breast tumors and 15 primary malignancies of the breast. Specific estrogen-binding capacity was found in 75% of pts with recurrent disease and in 60% of primary lesions, but of 23 benign breast tumors (13 fibrocystic disease, 4 fibrosis, 3 fibroadenoma, and 3 lipoma), only three contained ER. The ER concn in these were less than 10 femtomoles/mg cytosol protein, and intraductal papillomatosis, florid adenosis, or fibroadenoma were also demonstrated. The range of ER concn in the 20 recurrent lesions was from 2 to greater than 100 femtomoles/mg cytosol protein, while dissociation constants covered the narrow range of 6.8 x 10**-11 M to 5.6 x 10**-10 M. Regional lymph node and chest wall biopsies involved with tumor also showed wide range of ER concn. Negative results were obtained in five chest wall specimens. Primary tumors also varied widely with respect to ER content in 15 cases: negative results were obtained in 5 cases, eight ranged from 10-1-4 femtomoles/mg cytosol protein, and only one was less than 10 femtomoles. There were no significant correlations between estrogen-binding capacity and age or pathological findings. Menopausal age correlated in neither the primary nor the recurrent lesions. Because endocrine ablation is the therapy of choice in recurrent breast cancer, it is suggested that ER be assayed in all cases of recurrent breast cancer in order to predict a response to surgery and avoid an ineffective and unnecessary operation. (15 refs)

4. PROGESTERONE AND ESTRADIOL RECEPTORS IN HUMAN BREAST CANCER.

Huber PR, Geyer E, Wyss HI, Eppenberger U
Hormonlabor, Universitatsfrauenklinik, Schanzenstrasse 46, CH-4004 Basel

Experientia; 32(6):798 1976

Sera of 200 breast tumor patients have been screened for alterations in the level of Estradiol (E2), Progesterone (P), Prolactin and Growthhormone . Our results as well as those of

other workers have shown the plasma levels of above hormones to be normal in breast cancer patients. Therefore the reason for hormone dependency of breast carcinoma growth has to be looked for within the carcinoma cell itself. In our experiments we studied the content of estradiol receptors in 29 cases of human breast tumors and found that 40% contained the E2-binding protein. The receptor content was measured in 14 cases and 70% contained progesterone receptor. The tumor tissue which bind E2 and P had binding constants (Kd) of 0.04 nM and 0.03 nM respectively. In certain cases sucrose gradient studies carried out showed 4S and 8S binding for estradiol, which was completely abolished by U-11100 (an antiestrogen) indicating the specifity of E2-binding to both forms of the receptor. Similar experiments are presently carried out on the progesterone receptor using the highly specific progestin R5020 (Roussel-Uclaf). These experiments indicate that progesterone is bound to a component which is different to the estradiol receptor, but has similar properties. These findings suggest that not only E2 and its receptor have to be taken in account in hormonal therapy of human breast cancer but the progesterone receptor content as well. (Author Abstract)

5. CORRELATION BETWEEN ESTROGEN RECEPTOR PROTEIN AND CARCINOEMBRYONIC ANTIGEN IN NORMAL AND CARCINOMATOUS HUMAN BREAST TISSUE.

Menendez-Botet CJ, Nisselbaum JS, Fleisher M, Rosen PP, Fracchia A, Robbins G, Urban JA, Schwartz MK
Memorial Sloan-Kettering Cancer Center, Departments of Biochemistry, Pathology and Surgery, 1275 York Ave, New York, NY 10021
Clin Chem; 22(8):1366-1371 1976

We determined estrogen receptor protein and carcinoembryonic antigen in cytosols prepared from 189 human breast carcinoma tissues, 85 benign or normal breast biopsies, and 101 tissue specimens metastatic from breast carcinoma. Carcinoembryonic antigen was observed in 70% of the primary carcinomatous tissues, 15% of the benign or normal specimens, and 51% of the metastases. Ninety-six of the 189 primary carcinomatous specimens with increased concentrations of carcinoembryonic antigen were also positive for estrogen receptor protein, whereas 67 of the 72 benign or normal biopsies with low concentrations of carcinoembryonic antigen were also negative for estrogen receptor protein. All five benign specimens with positive estrogen receptor protein and normal carcinoembryonic antigen concentrations were from fibroadenomas. The concordance between estrogen receptor protein and carcinoembryonic antigen in the primary carcinomatous tissue was 66%, in metastatic carcinoma 51%, and in benign and normal tissue 85%. (Author Abstract)

6. THE PATHOLOGIC BEHAVIOR OF BREAST TUMORS: A REVIEW OF RECENT OBSERVATIONS. (PP. 421-431)

Dehner LP
Advances in Cancer Surgery. Najarian JS, Delaney JP, ed. New York, Stratton Intercontinental Medical Book Corp, 1976.

The natural history and morphology of carcinoma of the breast, its multicentricity, special histologic variants, and the relationship between estrogen receptors and breast cancer is reviewed. The morphologic appearance of carcinoma of the breast may be used to predict the natural history. Patients with the better differentiated or lower grade carcinomas had a significantly better survival (22% at 5 yr after diagnosis) than those with poorly differentiated tumors (0% at 5 yr). Those tumors still confined to their site of origin with an intact basement membrane have an excellent prognosis. Intraductal carcinoma with a solid or papillary growth pattern and lobular carcinoma in situ have an extremely favorable outlook. Well-differentiated adenocarcinoma, medullary carcinoma with or without lymphocytic stroma, pure mucinous carcinoma, and adenoid cystic carcinoma are all invasive neoplasms but have a lower frequency of nodal metastases. In a study of 157 breast cancers, there were 85 multifocal neoplasms, 58 of which were located in a different quadrant than the original lesion. Multicentric neoplasms occurred more often when the dominant mass was 5 cm or greater in diameter, involved the nipple, and had a prominent intraductal component. Nearly 70% of the multicentric tumors were noninvasive neoplasms with the majority classified as intraductal carcinomas. The single small cell pattern of infiltration and the arrangement about the foci of lobular carcinoma in situ are used in the diagnosis of invasive lobular carcinoma. An as yet unexplained association is the occurrence of lobular carcinoma in situ with fibroadenomas. Lipid-rich and squamous cell carcinomas of the breast are two uncommon histologic variants. The lipid-rich carcinoma has a tendency to metastasize to the orbit and eyelid. Focal squamous metaplasia or unequivocal squamous cell carcinoma may be recognized in an otherwise typical infiltrating duct carcinoma. Surveys of breast carcinomas indicate that 40-50% of neoplasms contain 8s and 4s protein receptors for 17 beta-estradiol in the cytoplasm. Similar receptor activity has been demonstrated in approx 15% of benign breast lesions. The principal significance of these receptors is that they provide an objective marker to identify those patients who may respond to endocrine ablative surgery if and when they develop metastatic carcinoma.

7. ESTROGEN RECEPTOR AND NATURAL COURSE OF BREAST CANCER.

Singhakowinta A, Potter HG, Buroker TR, Samal B, Brooks SC, Vaitkevicius VK
Milton A. Darling Memorial Center, Michigan Cancer Foundation, The Grace Hosp. 4160 John R St., Detroit, MI 48201
Ann Surg; 183(1):84-88 1976

Estrogen receptor (ER) was studied in 133 pts. (102 postmenopausal, 31 premenopausal) with breast cancer and related to the course of the disease. Tumors contained ER in 51% of all pts., in 45.2% of premenopausal pts., and in 53% of postmenopausal pts. The mean ER content in premenopausal pts was 23.4 femtomole (fmole), compared to 39.7 fmole in postmenopausal women. There was a preponderance of bone metastases in the ER-positive group, while in the ER-negative group, visceral metastases appeared dominant (p less than 0.0001). Among 12 pts who had multiple biopsies, 11 showed no significant discrepancy in ER from various sites of metastases. In three pts., ER was assayed during treatment with diethylstilbestrol (DES, 15 mg/d.). ER values prior to, during, and after DES in one pt were 3.3, 0, and 11.5 fmoles/mg protein, resp. The other two had ER assayed during and after DES, and ER in both rose after discontinuation of treatment: 0 to 2.2, and 4.8 to 61 fmoles/mg protein at 4 and 7 mo., resp., after discontinuation of DES. Among 90 pts in whom the disease-free interval could be measured, there was no significant difference between positive and negative ER groups. The mean survival in the ER-positive group was 56.7 mo., compared to 29.6 mo for the ER-negative group (p less than 0.01). It is suggested that study of ER should be done in all breast cancer pts., regardless of operability, not only as a guide to choice of therapy but also a prognostic indicator. (8 refs)

8. PATHOLOGICAL REVIEW OF BREAST LESIONS ANALYZED FOR ESTROGEN RECEPTOR PROTEIN.

Rosen PP, Menendez-Botet CJ, Nisselbaum JS, Urban JA, Mike V, Fracchia A, Schwartz MK

Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Cancer Res; 35(11/1):3187-3194 1975

An effort was made to relate estrogen receptor protein (ERP) levels to tissue morphology of specimens taken from 147 primary breast carcinomas, 78 metastases, 27 fibroadenomas, and 81 nonneoplastic tissues. The results showed a high frequency of positive ERP in invasive lobular carcinoma compared to typical ductal tumors and low frequency of positive ERP in tumors with a prominent local lymphocytic reaction. No statistically significant relationship existed, however, between ERP and any other morphological features examined.

9. ESTROGEN RECEPTORS IN HUMAN AND ANIMAL MAMMARY GLAND TUMORS.

El'tsina NV

Moscow, USSR

Vopr Onkol; 21(5):118-126 1975

The possibility of a correlation between estrogen receptor (ER) content in tumor tissue and hormone dependence was discussed. The relationship of the quantity of ER protein in the cytoplasm of tumor cells and their hormone-dependent growth was found to be disturbed. The growth of primary mouse mammary malignancies in ovariectomized and intact mice progressed at the same rate, independent of their receptor content. This might be ascribed to a factor in the course of development different from the interaction of estrogen and receptor protein. The presence of ER in the cytoplasm of malignant tissue was considered to be still insufficient proof to conclude a possible stimulating effect of a hormonal trigger on the genetic cell mechanism. The same view with regard to human breast tumors must be retained. A group of 1,184 patients stricken with breast carcinoma was studied. Only in 54% of the cases, a protein receptor binding estradiol to the tumor was found, which meant that almost half the patients' tumors were hormone-insensitive. The number and affinity of receptors to estradiol varied among the individual cancers. It was noteworthy that ER were not found in benign breast tumors. Here estrogen-binding was non-specific. The importance of ER studies for the differential therapy of mammary cancer metastasis is emphasized.

10. SIMPLIFIED SCATCHARD-PLOT ASSAY FOR ESTROGEN RECEPTOR IN HUMAN BREAST TUMOR.

Johnson RB, Nakamura RM, Libby RM

Scripps Clin. Res. Found., La Jolla, CA

Clin Chem; 21(12):1725-1730 1975

A 2-point Scatchard-plot assay including a test of competitive inhibition is described. After homogenization in a buffer containing 1.5 mM disodium ethylenediaminetetraacetate and 1 mM dithiothreitol, a fat-free cytosol is obtained by ultracentrifugation. Free and bound labeled estradiol are separated with dextran-coated charcoal after overnight incubation at 4C. Precision and reliability of the method were tested by assaying cytosols prepared from pregnant rabbit uteri before and after dilution with kidney cytosol and by assaying several other animal and human tissues, both target and nontarget. The Scatchard plot data were more reliable than tests for percent inhibition of competitive binding by diethylstilbestrol. A binding of at least 8 x 10**-15 mole of estradiol/mg protein is necessary for a tumor tissue to be considered positive, and the Kd must be in the range of 0.1-5 x 10**-10 mol/l. Seven of 19 breast tumor specimens were found to be positive. The method satisfies most of the requirements for the assay of estrogen-binding protein. Four tumor tissues and a positive and a negative control can be assayed in only 64 tubes with about 5 hr of technician time. (20 refs)

11. HORMONAL RECEPTORS OF MALIGNANT TUMORS OF THE BREAST.

Heuson JC, Longeval E, Leclercq G, Mattheiem WH

Serv. Med. Lab. Investigation Clin., Inst. Jules Bordet, Cent. Tumeurs Univ. Libre Bruxelles, Bruxelles, Belgique

J Gynecol Obstet Biol Reprod (Paris); 4(2):207-214 1975

The analysis of tissue samples from 166 primary and 136 metastasized breast cancers for the presence of estrogen receptors showed that, by measuring the affinity of the cytoplasmic fraction for ^{3}H-estradiol-17-b, receptors could be found in 72% primary cancers and 54% metastasized cancers. Receptor concentrations varied from 0 to 2,080 femtomoles/mg tissue protein. It thus seems that breast tumors differ quantitatively rather than qualitatively. Receptors were detected in mammary dysplasia, fibroadenomas, and in one papilloma, but not in non-neoplastic mammary glands, nipples, areola, or skin. Mastectomy revealed no relation between the presence or absence of receptors in primary forms and the presence or absence of metastases of the axillary nodes. There was a parallel between the presence or absence of receptors in the primary cancers and the presence of receptors in axillary metastases. Remission after endocrine treatment was obtained in 82/118 pts with estrogen receptors, compared to 8/134 in pts without receptors (pooled data from several reports). (12 refs)

12. THE PREDICTION OF HORMONAL DEPENDENCY OF MAMMARY CANCER.

Block GE, Jensen EV, Polley TZ

Dept. Surg., Univ. Chicago Hosp., Clin., Chicago, Ill.

Ann Surg; 182(3):342-352 1975

Results of estrogen receptor protein (estrophilinE) determination in predicting the hormonal dependency of mammary cancer are summarized. E was assayed in fresh or frozen specimens from 359 primary breast cancers and from metastatic tumors in 214 pts by addition of tritiated estradiol to the supernatant of the cytosol fraction of a tissue homogenate, followed by ultracentrifugation in sucrose gradients to demonstrate the 8S receptor protein; results were expressed as femtomoles (fmole) of estrogen bound per g of tumor. A positive E determination was defined as receptor content greater than 250 fmole/g for premenopausal women and greater than 750 fmole/g for postmenopausal or previously castrated pts. The median age of pts with receptor-negative primary tumors was 54 yr, significantly lower than that of pts with receptor-positive tumors (60 yr), but there was no correlation of receptor content with tumor size or histologic features, site of metastases, or clinical stage of disease. A total of 98 pts eventually received endocrine therapy (82 ablative treatment and 16 hormonal additive treatment); the records of 81 of these pts were evaluated by a peer review group to assess response to treatment, objective response requiring measurable remission in the absence of new or continued tumor growth over 6 mo. Among 69 evaluable pts undergoing ablative therapy, 42 had negative receptor determinations and none of these experienced objective remission; of the 27 pts with positive receptor determinations 13/19 evaluable pts could be rated as experiencing objective remission (9/14 treated by adrenalectomy with or without castration, 1/1 following hypophysectomy, and 3/4 treated by castration alone). Hormone additive therapy was beneficial in 4/6 pts with positive receptor determinations but only 1/6 with negative determinations. Estrogen receptor patterns were generally consistent in multiple specimens secured through the course of the disease; among 23 pts, 2 had positive tumors

giving rise to negative metastases, 1 had a positive tumor with positive and negative local recurrences, and 1 had a negative tumor with positive and negative chest wall recurrences, while the other 19 showed consistent patterns for intervals up to 67 mo, and endocrine therapy following development of metastases in 8 pts was successful in 2 whose primary tumors were receptor positive but failed in 6 whose primary tumors were receptor negative. It is concluded that the receptor characteristics of a primary tumor can guide subsequent endocrine therapy when metastases are inaccessible for receptor assay. (19 refs)

13. IN VIVO BLOCKADE OF THE ESTRADIOL-BINDING-PROTEIN (EBP) BY CLOMIPHENE CITRATE IN HUMAN BREAST CANCER.

Garola R, Levy CM, Vegh I, Magin C, Martinez JC, Hecker E
Endocrinol. Sect., Univ. Buenos Aires, Buenos Aires, Argentina
Oncology; 30(2):105-112,1975

The effect of clomiphene citrate (CC) admin in vivo upon in vitro uptake of labelled estradiol in a pt with significant amount of estradiol binding protein (EBP) bearing disseminated metastatic breast cancer is reported. The 60 yr old pt presented with multiple s.c metastatic nodules 10 mo after radical mastectomy. The pt was given CC at 200 mg/d. Prior to CC, the tumor cytosol assay indicated a significant amount of EBP. Addition of CC in vitro to the cytosol showed a strong competitive effect. This was correlated with a significant reduction in skin nodule size and number in the pt. After 26 d of treatment, biopsy material showed no significant binding of estradiol to cytosol protein and lack of competitive activity of estradiol with CC. (29 refs)

14. THE STEROID ALCOHOL AND ESTROGEN SULFOTRANSFERASES IN RODENT AND HUMAN MAMMARY TUMORS.

Godefroi VC, Locke ER, Singh DV, Brooks SC
Michigan Cancer Foundation, Detroit, Mich. 48201
Cancer Res; 35(7):1791-1798 1975

Rodent and human mammary tumor systems were investigated to relate the steroid alcohol and estrogen sulfotransferase activities to the hormonal dependency of the tumor as determined by estrogen receptor content. Unlike the normal mammary gland or the hyperplastic alveolar nodule, rodent mammary neoplasms displayed significant levels of these two sulfotransferases. In the hormone-independent mouse tumors produced from outgrowth lines D1, D2, and D8, high dehydroepiandrosterone sulfotransferase activity was characteristic of the rapidity with which hyperplastic alveolar nodules developed into a neoplasm (Vmax=52.8 vs 1.8fM/min/mg protein) while estrone sulfotransferase activity was either not detectable or low (Vmax=5.5fM). After oophorectomy of BALB/c mice bearing slowly developing tumors, both sulfotransferases in the nonregressing neoplasms showed marked increases in activity (Vmax dehydroepiandrosterone=30.0fM; Vmax estrone= 18.5fM). Strain differences were found to influence the steroid-sulfating capability but not the estrogen receptor content of hormone-dependent rat mammary tumors. In Wistar-Lewis rats the steroid alcohol sulfotransferase activity was at least 35 times higher than in the Sprague-Dawley strain. As was observed in the mouse mammary tumor, Sprague-Dawley rat neoplasms that grew in the absence of ovarian hormones contained significantly greater levels of the steroid alcohol sulfotransferase. A possible correlation between presence of the steroid alcohol sulfotransferase and the estrogen receptor protein was observed in a limited number of human breast carcinomas. A positive clinical response of four patients to hormonal manipulation was matched in all four cases with the presence of estrogen receptor protein and in three with the presence of the steroid alcohol transferase. The data support the concept that the sulfotransferase pattern of breast tumors may be related to hormonal dependency of the neoplasm and to the eventual prognosis of the disease.

15. HIGH AFFINITY OESTRADIOL RECEPTORS AND THE ACTIVITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND LACTOSE SYNTHETASE IN MAMMARY CARCINOMATA OF POSTMENOPAUSAL WOMEN.

Daehnfeldt JL, Schulein M
Fibiger Lab., Copenhagen, Denmark
Br J Cancer; 31(4):424-428 1975

Development of predictive tests for hormone dependence in human mammary tumors is of considerable importance in planning rational endocrine therapy for patients with breast carcinoma. In 26/57 patients at least 6 mo post menopausal, less than 2 x 10**-14 M estradiol was bound/mg protein. In 31/57 patients considered to be receptor positive, greater than 2 x 10**-14 M estradiol was bound/mg protein; 18/31 patients showed an estradiol binding capacity greater than 10 x 10**-14 M/mg protein. The glucose-6-phosphate dehydrogenase (G-6-PDH) activity was significantly higher in the receptor positive patients than in the receptor negative ones. The activity of lactose synthetase A (LSA) was also significantly higher in the receptor positive patients than in the receptor negative ones. The high activities of G-6-PDH and LSA found in tumors with high contents of estradiol receptor protein indicate that the receptor protein in these tumors is functional at the estrogen concn present in the patients (21 refs)

16. INFLUENCE OF ANTI-OESTROGENS ON THE SPECIFIC BINDING IN VITRO OF (' H) OESTRADIOL BY CYTOSOL OF RAT MAMMARY TUMOURS AND HUMAN BREAST CARCINOMATA.

Powell-Jones W, Jenner DA, Blamey RW, Davies P, Griffiths K
Welsh Natl. Sch. Med., Heath Park, Cardiff, Wales
Biochem J; 150(1):71-75 1975

The anti-estrogenic potential of 2 compounds containing nitrogen-mustard groupings, I.C.I. 79792 and I.C.I. 85966, was evaluated by their comparative ability to inhibit specific binding of ' H-estradiol by cytoplasmic proteins of human breast tumor and rat mammary carcinomas induced by 7,12-dimethylbenz(a)anthracene. Analysis of ' H-labeled cytosol preparations from rat mammary tumors showed 2 peaks of protein-bound radioactivity corresponding to estradiol-receptor complexes of sedimentation coefficients 4S and 8S. Low capacity of the 8S peak was demonstrated by the displacement of radioactivity to the high-capacity 4S peak by the presence of a 100-fold excess of unlabeled estradiol. Although either diethylstilboestrol (50 DE; nM) or dibutyldihydrostilboestrol (50 DB; nM) produced an identical displacement, only DE caused some depression of the 8S peak at 5nM concn. I.C.I. 85966 failed to diminish ' H-estradiol binding to the 8S receptor; I.C.I. 79792 (50nM) was not as effective as I.C.I. 46474 at the same concn. In 2 of the rat mammary-tumor cytosols studied, I.C.I. 85966 was more effective than I.C.I. 79792 and decreased specific binding of ' H-estradiol almost as efficiently as DE, DB and I.C.I. 46474. This effect may be due to differing affinities of receptors for estrogen and anti-estrogens in different tumor cytosols, or to an independent factor. (17 refs)

17. STEROID HORMONE RECEPTORS IN HUMAN BREAST CANCER AND CLINICAL CORRELATIONS.

Maass H, Engel B, Nowakowski H, Stolzenbach G, Trams G
Universitats-Frauenklinik, Hamburg, W. Germany
Arch Geschwulstforsch; 45(5):423-429 1975

Data are presented on the correlation of clinical course

with estrogen receptor status in human breast cancer. Estradiol binding was assayed by uptake of H-17-b-estradiol in tissue slices or by identification of the estradiol-receptor complex on agar gel electrophoresis of the cytosol. Estradiol receptors were found in 36 of 72 premenopausal pts. (50%) and 103 of 220 postmenopausal pts. (47%). The incidence in cases of metastatic disease was 57 of 168 (34%). There was no correlation with stage of disease or histologic features, except that dedifferentiation was more frequent in receptor-negative tumors. Objective remissions (over 12 mo) were observed in 18 of 35 receptor-positive pts following endocrine therapy (castration, hypophysectomy, ethinyl E2 , androgens, or antiestrogens) but in only 3 of 43 receptor-negative pts. (all treated with nafoxidine). In particular, remissions were seen in 5 of 6 receptor-positive pts undergoing ovariectomy, but in 0 of 19 ovariectomized receptor-negative pts. In the receptor-positive group there was no difference in quantitative receptor content between responders and nonresponders, and the duration of remission was independent of the receptor level. (10 refs)

18. METABOLISM OF DEHYDROEPIANDROSTERONE BY HORMONE DEPENDENT AND HORMONE INDEPENDENT HUMAN BREAST CARCINOMA.

Abul-Hajj YJ
Coll. Pharm., Univ. Minn., Minneapolis
Steroids; 26(4):488-500 1975

An investigation of dehydroepiandrosterone (DHA) metabolism by human breast carcinoma tissue in vitro is described. Tumor tissue (2 g) from six pts. (aged 52-69 yr) was incubated with 25 uCi of 7a**-3 H-DHA for 2 hr, and steroid metabolites were separated by thin layer chromatography. All tumors transformed DHA to testosterone, dihydrotestosterone, 5a-androstane-3a, 17b-diol, and 5a-androstane-3,17-dione. In addition, estradiol was produced by 4 tumors, only 1 of which was estrogen receptor-positive, based on measurement of dissociation constants for binding of estradiol to receptor protein. No conversion to estrone was detected. It was suggested that the capacity of estrogen receptor-negative tumors to produce estradiol may explain why these hormone independent tumors are unresponsive to hormone therapy and endocrine ablation, since the tumors may provide their hormonal stimulation (including estradiol) by de novo synthesis of sex hormones at the tumor level. Moreover, the unresponsiveness of certain estrogen receptor-positive (hormone dependent) tumors may be explained by the observed capacity of one such tumor to convert C-19 steroids to other androgen metabolites as well as estradiol. (19 refs)

19. SIGNIFICANCE OF TISSUE ESTROGEN RECEPTORS IN THE STRATEGY OF BREAST CANCER TREATMENT.

Heuson JC, Leclercq G
Service Medicine Laboratoire d'Investigation Clinique Institut Jules Bordet, Centre Tumeurs Universite Libre Bruxelles, Brussels, Belgium
Arch Geschwulstforsch; 45(5):417-422 1975

Estrogen receptors were detected in 73% of 214 primary breast cancers and 58% of 168 metastatic tumors. Improved techniques for determining the receptors caused the rate of receptor positive tumors to reach 82% and 70% in the second half of the determinations made in this series. These results were believed to be closer to reality than the overall percents. Receptor concn varied over a wide range (2,080 femtomoles/mg tissue protein to 0). A tumor and its closest metastases contain the same concn of receptors. Clinical applications of estrogen receptors in breast cancer include: possible compounds that bind to estrogen receptors and inhibit tumor growth by interfering with their metabolic activity, prediction of response to endocrine treatments according to presence or absence of receptors, and a new therapeutic strategy. Work on possible therapeutic compounds, previously reported, was reviewed. A clinical trial of Nafoxidine has indicated its efficacy. A clinical trial of phenol-bis-(2 - chloroethyl) carbamate of estradiol (Estracyt) showed lack of efficacy. Results of a previous study of correlation between presence or absence of receptors and response to endocrine treatments were reviewed. The objective remission rates in pts with receptor-positive and receptor-negative tumors were 25% and 7% resp. This response for receptor-positive pts was low; however, when the quantitative relationship between receptor content of a tumor and the pt response was analysed, the mean receptor concn was significantly higher in the responders than in the non-responders. It is necessary to establish threshold values of receptor content above which endocrine therapies have a reasonable ance to be effective. The traditional therapeutic approach in breast cancer (endocrine treatment followed as a last resort by cytotoxic chemotherapy) can be guided by estrogen receptor analysis. The level of receptor concn will indicate the probable degree of hormone dependence. A different approach suggested is to use endocrine treatment in all cases, complemented at once by cytotoxic therapy as a combined hormonal-cytotoxic approach. Recently devised modalities of this type have proved quite effective with an acceptable level of general toxicity. (24 refs)

20. CLINICAL TRIAL OF CLOMIPHENE IN ADVANCED BREAST CANCER.

Hecker E, Vegh I, Levy CM, Magin CA, Martinez JC, Loureiro J, Garcia RE
Buenos Aires Univ., Oncol. Inst., Endocrinol. Sect., Buenos Aires, Argentina
Eur J Cancer; 10(11):747-749:1974

The effect of clomiphene citrate was investigated in 50 postmenopausal women and one man with advanced breast cancer. Pts were admitted to clinical trial if they fulfilled the following criteria: Stage IV (UICC) breast cancer; histological diagnosis of the disease; postmenopausal status either natural or surgical of at least one yr; and free of any specific cancer treatment for at least 3 mos. The drug was admin po at 2 dose levels:200 mg/d. (43 pts) or 300 mg/d. (8 pts) for at least 60 d unless unequivocal progression of the disease occurred. Side effects were mild and no pts were withdrawn from therapy; nausea was the most common side effect. Each pt had a clinical control every 3 wks and complete bone survey and chest X-ray examination every 6 wks. Objective remission was found in 20 pts; 13/31 non-responders showed either arrest or objective regression in some metastases. Mean length of remission was 12 mos. No difference in response was observed among pts treated either with 200 or 300 mg of the drug. Clomiphene citrate is able to block in vitro the uptake of labelled estradiol by estradiol binding protein. Its admin also reduced the number and size of DMBA-induced mammary cancer of the rat. These studies indicate clomiphene citrate to be of therapeutic value in advanced breast cancer in that 39% of the pts treated experienced objective remission. (16 refs)

21. ESTROGEN RECEPTORS IN BREAST CANCER AND ENDOCRINE THERAPY.

Maass H, Trams G, Nowakowski H
Univ. Gynecol. Clin., Hamburg-Eppendorf, West Germany
Strahlentherapie; 147(4):381-387 1974

Estrogen receptors in tumor tissue were determined in 225 patients with primary breast carcinoma and in metastatic tissue of 120 cases, to discover whether a correlation existed between the presence of such receptors and the course of the disease following endocrine therapy. Objective remissions were obtained in 12/23 patients in whose tumor tissue estrogen receptors were found and who were treated by ovariectomy, hypophysectomy, estrogen, Metenolon or antiestrogen. Only 2/32 patients had objective remissions in the group that gave negative results for estrogen receptors in their tumor tissue,

and who were given endocrine therapy; 2/5 patients who received Nafoxidin. Estrogen receptors were found in tumor tissue in 45% of patients with primary breast carcinoma, and in metastatic tissue in 40% of the advanced cases. Serum estradiol levels, determined simultaneously with tissue examination revealed that the free cytoplasmic receptor content varied inversely with these levels. To prevent the occurrence of false negative results, it is therefore recommended that the estradiol conc. (in serum) be determined as well as the estrogen receptors in the tissue. (24 refs)

22. CORRELATIONS BETWEEN CERTAIN BIOCHEMICAL PROPERTIES OF BREAST CANCER AND RESPONSE TO THERAPY: A PRELIMINARY REPORT.

Savlov ED, Wittliff JL, Hilf R, Hall TC
Univ. Rochester Sch. Med. Dent., Rochester, N.Y.
Cancer; 33(2):303-309 1974

Tumor tissue from 130 patients with primary or metastatic breast cancer and tissue from 104 patients with benign breast lesions were assayed for estrogen-binding proteins (estrogen receptors) and for several enzymes involved in carbohydrate and lipid metabolism (glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase, pyruvate kinase, glucose phosphate isomerase, and alpha-glycerol phosphate dehydrogenase). Pts were treated by a variety of modalities, including ablative hormonal therapy (bilateral oophorectomy and/or bilateral adrenalectomy), additive hormonal therapy (diethylstilbestrol 15 mg/d, po) or fluoxymesterone 30 mg/d, po) or chemotherapy (single or multiple agents, no further details). Normal breast tissue, fibrocystic disease, and fibroadenomas usually exhibited lower enzyme levels than malignant disease and rarely contained specific estrogen-binding proteins. Biochemical characteristics unique to intraductal carcinoma that may be of value in the prediction of response to various types of therapy are discussed. A bar graph showing the relationship of activities of the various enzymes and the presence or absence of estrogen binding capacity in infiltrating duct carcinomas is presented. Chemotherapy response seemed to be independent of estrogen-binding capacity; roughly equal numbers of patients with or without estrogen receptors responded to chemotherapy with antimetabolites or alkylating agents. High levels of glycolytic enzymes may predict a response to chemotherapeutic agents. (24 refs)

23. BIOCHEMICAL BASIS FOR STEROID ANTICANCER ACTION.

Terenius L
Dept. Med. Pharmacol., Univ. Uppsala, Uppsala, Sweden
Progress in Chemotherapy; Volume III: Antineoplastic Chemotherapy: Proceedings of the 8th International Congress of Chemotherapy Athens, 1973 George K. Daikos, Editor. Hellenic Society for Chemotherapy, Athens:329-334 1974

The presence or absence of steroid hormone receptors may determine whether tumor cells will respond to some kind of hormone therapy. Estrogen receptors were found to be present in 35-50% and progesterone receptors in 30% of tested human breast tumors. Whenever a primary tumor had receptors, the secondary metastases usually also had them. The presence of receptors did not correlate with the state of differentiation, the histopathology, or the cellularity of the tumors. Only 10% of benign tumors had receptors. The tissue surrounding tumors lacked receptors. Hormone-dependent tumors regress after deprival of a hormonal stimulus and such deprival may occur via the pharmacologic suppression of hormone biosynthesis, or via the competitive blocking of receptors by antihormones, such as nafoxidine. One problem in steroid therapy is that responsive tumors often progress to non-responsiveness, presumably through the loss of the capability to make receptor protein. (33 refs)

24. ESTROGEN RECEPTORS IN HUMAN BREAST CANCER.

McGuire WL
Univ. Tex. Med. Sch., San Antonio
J Clin Invest; 52(1):73-77 1973

Sucrose gradient centrifugation was used to quantitate estradiol-binding protein (EBP) in both primary and metastatic human breast cancer tissue; EBP was present in a broad range of concn from zero to very high levels expressed as estradiol-binding sites/mg cytoplasmic protein. Earlier published data, obtained from less sensitive methodology, had suggested EBP was present in an "all or none" fashion. Since data from animal breast tumors and preliminary evidence from human breast tumors indicate an excellent correlation between presence of abundant tumor EBP and endocrine-induced regression, quantitation of EBP may prove to be an excellent prognosticator of endocrine therapy in metastatic breast cancer. (19 refs)

25. STUDIES ON THE ESTROGEN RECEPTOR IN BREAST CANCER.

McGuire WL, Chamness GC
Univ. Tex. Med. Sch., San Antonio
Adv Exp Med Biol; 36:113-136 1973

In estrogen-dependent rat mammary tumors induced by dimethylbenzanthracene, the cytoplasmic estrogen receptor (CER) was excluded by Sephadex G-100, sedimented at 8S in low salt and 4S in high salt sucrose gradients, exhibited very high affinity binding of estradiol, and displayed stringent structural ligand requirements. In concn. 1,000-10,000x greater than that of added ^{3}H-estradiol, the nonestrogenic steroids hydrocortisone, progesterone, and testosterone failed to inhibit this binding of estradiol. In the R323OAC transplantable rat mammary carcinoma (autonomous), which does not regress after ovariectomy, the nuclear fraction of the tumor did not accumulate estradiol, and the capacity of estrogen binding sites in the cytoplasm was only 2.4 "femtomoles"/mg cytosol protein, compared to 26.0 "femtomoles"/mg cytosol protein in the estrogen-dependent tumor. Cell-free studies demonstrated that the chromatin of autonomous tumor nuclei was able to bind CER from exogenous sources. CER from human breast cancer was excluded by Sephadex G-100, sedimented at 8S and 4S, complexed with protamine, and did not bind progesterone, testosterone, hydrocortisone, or 5beta-dihydrotestosterone. The capacity of estrogen binding sites was 0 in 18/40 patients with primary mammary carcinoma and ranged from 0.6 to 612 "femtomoles"/mg cytosol protein in 22/40 patients; in 7/21 patients with metastatic mammary carcinoma, the capacity for binding was 0, and it ranged from 1.8 to 185 "femtomoles"/mg cytosol protein in 14/21 (biopsy sites: breast, lymph node, ovary, liver, or bone). It is concluded that the percentage of cells in a tumor that contain CER can vary from 0 (all CER-) to 100% (all CER+) and that CER+ cells will regress after hormone therapy, whereas CER- cells will not. It is proposed that CER assays should be done on all patients undergoing mastectomy for breast cancer; approx half the patients will return with metastatic disease, and reassay for CER should be done if the lesion is accessible. On the basis of this assay, chemotherapy would be indicated in patients with negative primary tumor or metastatic tumor, whereas endocrine therapy would be indicated in patients with positive metastatic tumor or (when the metastatic lesion cannot be assayed) in patients with positive primary tumor. (13 refs)

26. HORMONAL DEPENDENCY OF BREAST CANCER.
Jensen EV, Block GE, Smith S, DeSombre ER
Recent Results Cancer Res; 42:55-62 1973

Results reported for this study indicate that in vitro determination of the estrogen receptor content of specimens of breast tumor can be used to predict a priori which breast cancers are hormone-dependent and therefore responsive to endocrine ablation. Of one group of 117 primary tumors studied, 56 (45 with metastases) contained estrogen receptors, 53 (11 with metastases) did not, and 8 (3 with metastases) were borderline. The median age for the 56 patients with positive tumors was 63 yr (range 34-83), as compared to a median of 56 yr (range 32-86) for those with negative tumors. In another group of 57 metastatic tumors studied, 18 were positive and 34 were negative; median ages of these patients were 52 yr (range 32-69) and 51 yr (25-71), resp. Of the total of 81 patients with metastatic disease in the 2 groups, 54 received some type of endocrine therapy, and their clinical response was evaluated: of 32 patients whose tumors showed negative patterns, only 1 achieved remission after adrenalectomy, in contrast, 13/17 whose tumors contained receptors achieved objective remission. These studies show that in the case of metastatic breast cancer, most (but not all) patients with receptor-containing tumors may benefit from endocrine therapy, but if the tumor shows no evidence of estrogen receptors there is little chance of remission following endocrine ablative therapy. (24 refs)

27. CONTENT OF ESTROGEN RECEPTORS IN HUMAN BREAST CANCERS.
Spaeren U, Clsnes S, Brennhovd I, Efskind J, Pihl A
The Norwegian Radium Hosp., Montebello, Oslo
Eur J Cancer; 9(5):353-357 1973

The estrogen binding capacity of 251 human breast cancers has been determined. The cytosol of the tumors was incubated with labelled estradiol-17-beta and subjected to sucrose-gradient-density centrifugation. The difference in the radioactivity in the 8S area between samples incubated in the absence of an estrogen antagonist was taken as a measure of the estrogen receptor content. The receptor content of the tumors varied over a wide range. Measurable amounts were present in 184 cases (73.1%). The content was 2-3 times higher in tumors from patients above 50 yr than in those below this age. No relationship was found between the receptor content and the histological type of the tumors with the exception that tumors of the scirrhus type contained less receptors that other types of tumor. The receptor concentration was the same in different clinical stages of the disease suggesting that the receptor content of human breast cancers does not tend to decline with progression of the tumors.

28. STUDIES ON CERTAIN CYTOPLASMIC ENZYMES AND SPECIFIC ESTROGEN RECEPTORS IN HUMAN BREAST CANCER AND IN NONMALIGNANT DISEASES OF THE BREAST.
Hilf R, Wittliff JL, Rector WD, Savlov ED, Hall TC, Orlando RA
U. Rochester Sch. Med. Dentistry, New York
Cancer Res; 33(9):2054-2062 1973

Samples of normal breast tissue, fibrocystic disease, fibroadenomas, and infiltrating ductal carcinomas were examined for several cytoplasmic enzymes and specific estrogen-binding capacity, as well as for the levels of RNA, DNA, and protein. Compared with normal breast tissue, the activities per mg DNA of pyruvate kinase, glucose 6-phosphate dehydrogenase, isocitrate dehydrogenase, hexokinase, glucose phosphate isomerase, and aspartate aminotransferase were significantly elevated in carcinomas. Furthermore, the activities of these enzymes were significantly higher in the carcinomas, compared with those of samples of fibrocystic disease and fibroadenoma. Neither the age of the patient at the time the tumor was removed nor the presence of metastatic lesions was apparently related to the biochemical characteristics, although the carcinomas from women 70 years or older showed somewhat higher activities of glucose phosphate isomerase, lactate dehydrogenase, and glucose 6-phosphate dehydrogenase. Carcinomas that possessed specific estrogen-binding capacity, less than 7 fmoles (10-15 moles)/mg protein as estimated by sucrose gradient centrifugation of tumor cytosols, had lower activities of glucose 6-phosphate dehydrogenase, isocitrate dehydrogenase, and pyruvate kinase and slightly higher levels of DNA. The data reported here indicate that infiltrating ductal carcinoma of the breast presents with certain metabolic alterations that are not shared by nonmalignant breast diseases. Carcinomas that are potentially hormone responsive demonstrate a different enzyme activity profile from that of tumors that are potentially hormone independent.

29. SIMILARITY OF THE ESTROGEN RECEPTORS IN HUMAN AND RAT MAMMARY CARCINOMA.
McGuire WL, Delagarza M
Dept. Med., U. Texas, San Antonio
J Clin Endocrinol Metab; 36(3):548-552 1973

The properties of human and rat mammary tumor cytoplasmic estradiol binding protein (EBP) have been compared. Sephadex column chromatography of human tumor cytosol preparations preincubated with estradiol showed that human tumor EBP, as had previously been shown for rat tumor EBP, could be demonstrated by this method. Low-salt sucrose density gradient centrifugation of human tumor cytosol preincubated with 3H estradiol revealed radioactive peaks migrating as 8S and 4S particles. All the label in the 8S peak and most of the label in the 4S peak were eliminated by competition with unlabeled estradiol, indicating that both peaks contained EBP. These properties were identical to those of rat mammary tumor EBP. Like rat tumor EBP, human mammary tumor EBP was precipitated by protamine sulfate. Human tumor EBP binding was specific for estrogens as progesterone, testosterone hydrocortisone and dihydrotestosterone failed to compete significantly with estradiol.

30. ESTROGEN RECEPTORS IN HUMAN BREAST CANCER.
Leclercq G, Heuson JC, Schoenfeld R, Mattheiem WH, Tagnon HH
Jules Bordet Inst., Tumor Ctr. Free U. Brussels, Belgium
Eur J Cancer; 9(9):665-673 1973

Samples from 77 primary and 65 metastatic human breast cancers were assayed for specific cytoplasmic estrogen receptors. Cytosol preparations were incubated with increasing amounts of 3H-estradiol-17beta. The unbound radioactivity was removed by charcoal-coated dextran. Saturable high affinity binding sites were detected in 56% of the primary and 37% of the metastatic tumors. The binding constants of these receptor sites for estradiol were quite variable; most ranged from 1.0 to 20 X 10-10M, but some were larger (up to 108 X 10-10M). The concentrations of binding sites were distributed within a continuous range from 5 to 1330 femtomoles per mg protein. This wide range was not ascribable only to variations in amounts of contaminating serum proteins. Receptors were detected in only 8% of cytosol preparations containing less than 2 mg protein per ml; in contrast, they were detected in 53% of cases with higher protein concentration. This indicates that at low protein concentration, false negative results are likely to occur. Detectable amounts of receptors were not found in sera or samples from normal mammary gland, nipple, areola, skin,

and non-invaded lymph nodes. The reliability of a simplified procedure based on isotopic dilution of the labelled estradiol, to be used with very small tumor tissue samples, was studied. It compared quite well with the other one except in cases of very low concentrations of receptor. No relationship was found between the occurrence of receptor and the age of the patient or the histological type of the tumor. There was also no relationship between the occurrence of receptors in the primary tumor and presence or absence of metastatic axillary lymph nodes. However, when present, the latter had the same characteristics with respect to the receptor as the corresponding primary, with only one exception.

31. ABNORMAL ESTROGEN METABOLISM AND TISSUE ESTROGEN RECEPTOR PROTEINS IN BREAST CANCER.

Lemon HM
U. Nebraska Coll. Med., Omaha
Cancer: 25(2):423-435 1970

Estriol (E) significantly inhibited estradiol binding to tissue homogenates of dimethylbenzanthracene-induced rat mammary tumors. E also failed to prove carcinogenic in normal rats. In patients with breast cancer or chronic cystic disease of the breast, urinary E excretion tended to be subnormal, although E levels were normal or elevated in 5/6 women with a variety of cancers of other sites. It is concluded that estradiol and the "impeded" estrogens, including E, compete for those cytoplasmic proteins in the uterus, vagina and breast which are implicated in the initial concn of C 18 steroids, thus possibly influencing the risk of developing breast cancer. It is also suggested that E may play a significant anticarcinogenic role in women and may eventually prove to be an effective agent in the treatment of at least some hormone-dependent breast cancers.

32. CORRELATION BETWEEN URINARY STEROIDS AND ESTROGEN RECEPTOR CONTENT IN WOMEN WITH EARLY BREAST CANCER.

Hajj YJ
Coll. Pharmacy, Univ. Minnesota, Minneapolis, MN 55455
Eur J Cancer: 13(7):749-752 1977

The urinary levels of 11-deoxy-17-ketosteroids and estrogen receptor content in tumor tissues were determined in 76 patients with early breast cancer. Twenty-six postmenopausal and 16 premenopausal patients with estrogen receptor negative tumors were found to excrete subnormal amounts of 11-deoxy-17-ketosteroids while 22 postmenopausal and 12 premenopausal patients with estrogen receptor containing tumors excrete near normal levels of urinary 11-deoxy-17-ketosteroids. (Author abstract) (16 Refs)

33. ESTROGEN RECEPTOR IN BREAST CANCER OF THE JAPANESE.

Nomura Y
Dept. Breast Surgery, Natl. Kyushu Cancer Center Hosp., 595 Notame, Minami-ku, Fukuoka 815, Japan
Gann: 67(5):703-711 1976

The role of estrogen receptor (ER) in the incidence of breast carcinoma in Japan and as an index of ablative endocrine therapy was analyzed. There were 243 tissue samples from human breast cancer, benign breast disease, normal breast tissue, normal uterine myometrium and uterine myoma. The ER was positive in 52% of 96 patients with primary breast carcinoma (BC) and 54% of 24 tumors of metastatic BC. The binding sites ranged from 0-2,900 femtomoles/mg of cytosol protein in primary BC, which range was similar to normal myometrium, and 0-140 femtomoles/mg in metastatic BC. ER was present in 38% of benign breast tumors (fibroadenoma, chronic mastitis), with about 10% of macroscopically healthy breast tissue also positive; specimens of uterine myoma were 78% positive. The premenopausal, menopausal, and 5-yr post-menopausal patients with BC were 50, 56, and 50% positive, although the mean number of binding sites was greater in the post-menopausal group. A significantly higher av number of binding sites was found in the follicular phase when serum estradiol was low than in the luteal phase when estradiol was high. If the primary tumor contained ER, the metastatic tumor also bound estradiol. Recurrent metastases also contained positive ER with the same order of binding sites as the original. Metastatic ER were also relatively equally distributed, with 50% in skin, 60% in nodes, and 50% in liver. Patients with advanced carcinoma received initial hormone therapy before mastectomy. Of 14 estradiol-positive patients, 8 had tumor regression of at least 50% in 4-6 wk, 5 had decreases of less than 50%, and 4 progressed. Tumor regression of at least 6 mo occurred in 10/16 ER-positive patients with recurrent or metastatic breast cancer, with 1/16 failures. In 15 ER-negative patients, there were 14 failures and no successes. If the ER is absent, the probability of endocrine-induced tumor regression is low. (15 refs)

34. EFFECT OF DROSTANOLONE PROPIONATE ON THE BINDING OF OESTRADIOL AND DIHYDROTESTOSTERONE BY NORMAL AND MALIGNANT TARGET TISSUES.

Trams G
Dept. Obstetrics and Gynecology, Univ. Hamburg, Hamburg-Eppendorf, W. Germany
Eur J Cancer: 13(2):149-153 1977

The influence of the anticancer agent drostanolone propionate on the binding of 17b-oestradiol and 5a-dihydrotestosterone (5a-DHT) to specific receptor proteins in tissue from normal and neoplastic target organs was investigated. Aliquots of cytosols prepared from calf uterus or human breast cancer tissue were incubated with 4 x 10**-9 M ^{3}H-oestradiol in the presence or absence of Nafoxidine (2 x 10**-6 M) or drostanolone (5 x 10**-6 M). Agar gel electrophoresis of the labeled extracts revealed that inactivation of the binding protein by heat (48 C for 60 min) or displacement of the labeled oestradiol by the anti-oestrogenic compound Nafoxidine resulted in a disappearance of the receptor-bound labeled hormone and in an increase of the cathodical peak by the liberated steroid. In contrast, drostanolone did not compete at the oestrogen specific binding sites either in calf uterus or in human breast cancer tissue. When the cytosols of these tissues were incubated with 4 x 10**-9 M ^{3}H-5a-DHT in the presence or absence of cyproterone acetate (4 x 10**-6 M) or drostanolone (4 x 10**-6 M) and assayed for their androgen binding capacity, a clear discrimination between receptor protein and the sex hormone binding globulin (SHBG) from the serum was noted, with the latter binding 5a-DHT and migrating towards the cathode. The addition of a 1,000-fold excess of the anti-androgen cyproterone acetate or of drostanolone resulted in a decrease of radioactivity at the anodical peak which was due to competition at the specific receptor sites. This competition was less pronounced for SHBG by cyproterone acetate than for drostanolone. An electrophoretic pattern of a cutaneous metastasis derived from a patient with metastatic breast cancer who showed remission after drostanolone treatment revealed no 5a-DHT binding. Assay of the tumor revealed the presence of oestrogen receptors. The lack of 5a-DHT binding was possibly due to a primary lack of androgen receptors or to a depletion of spare 5a-DHT-receptor sites by the abundance of circulating drostanolone. Drostanolone had been given in a dosage of 100 mg/wk x 3 over a period of 17 mo up to 7 days prior to excision of the tumor. It appears unlikely that the growth

inhibitory effect of drostanolone in human breast cancer is mediated through interaction with oestradiol binding proteins. (19 refs)

35. PHASE II STUDY OF TAMOXIFEN: REPORT OF 74 PATIENTS WITH STAGE IV BREAST CANCER.

Lerner HJ, Band PR, Israel L, Leung BS
Dept. Surgery, Pennsylvania Hosp., Philadelphia, PA 19107
Cancer Treat Rep; 60(10):1431-1435 1976

The efficacy and toxicity of Tamoxifen (NSC-180973) was studied in 74 women with metastatic (Stage IV) breast adenocarcinoma. All but 3 patients were postmenopausal. Thirty-one patients were treated with 10 mg bid (approximately 6 mg/meter^2 bid) and 43 received 15 mg/m^2 bid. Treatment was given for 16 wk or until sign of disease progression or toxicity. Prior to therapy, tumor samples were taken and tested for estrogen receptors (ER) using a dextran-coated charcoal assay method. Tumors with estrogen binding sites greater than 1 femtomole/mg of cytoplasmic protein were considered ER positive. Of the 31 patients receiving 10 mg bid, 30 were treated greater than 4 wk. Of these, 5 complete and 11 partial responses were obtained. The median duration of response was 312+ days. The median age of responders was 53 yr vs 60 yr for nonresponders. Of those patients tested for estrogen receptors, 9/13 ER positive patients responded to tamoxifen, 2 had disease progression and 2 remained stable. Response was seen in 7/9 patients with soft tissue lesions, 2/16 with bone and 7/15 with visceral lesions. Of those with ER negative tumors, 3/4 progressed and 1 remained stable. Of the 45 patients receiving 15 mg/m^2 bid, 44 were treated greater than 4 wk. Of these, 3 complete and 16 partial responses were noted for a median duration of 227+ days. Response was noted in 7/11 patients with soft tissue lesions, 5/12 with bone and 7/21 with visceral lesions. The median age of responders was 56 yr (vs 58 yr for nonresponders). In general, toxicity was mild consisting mostly of hot flashes (23/74), transient leukopenia (11/74), transient thrombocytopenia (7/74), nausea (4/74) and fluid retention. Only one patient required discontinuation of therapy. Temporary dosage reduction was required for another patient due to severe bone pain. It is concluded that tamoxifen is an effective single agent with minimal toxicity for treatment of metastatic breast cancer. Estrogen receptor assay appears to be valuable in selecting patients for tamoxifen treatment. (10 refs)

36. LOW DOSE TAMOXIFEN IN THE TREATMENT OF METASTATIC BREAST CANCER AT THE PENNSYLVANIA HOSPITAL (MEETING ABSTRACT).

Lerner H, Godwin MC, Wach BA, Trench L
Pennsylvania Hosp., 8th and Spruce Sts., Philadelphia, PA
Int J Radiat Oncol Biol Phys; 2(Suppl1):78 1977

Tamoxifen (NIH:NSC 180973), is a synthetic non-steroidal compound with potent anti-estrogen activity. This study was designed to determine the efficacy of tamoxifen as a single agent in the treatment of metastatic breast cancer. Where possible, responses were correlated to the presence or absence of estrogen receptor protein. Patients were treated with 10 mg tamoxifen tablets twice daily for 8 wk. If there was regression or no change, treatment was continued until there was progression. Tamoxifen was well tolerated by the entire group. Hot flashes occurred in 15 patients as transient leukopenia in 6 patients and transient thrombocytopenia in 4 patients. Thirty-one patients were entered into the study. One patient with pleural effusion was considered non-measurable disease and three patients with insufficient treatment were excluded. Of the 27 evaluable patients there were 5 complete and 11 partial responses. The median duration of response was 312+ days. Responses were seen in soft tissue, visceral and bone metastases (with healing of bone metastases). Seventeen patients had analysis performed for estrogen receptor protein (ER): 12 patients were ER positive and 5 were ER negative. Nine of the ER positive patients responded to tamoxifen. None of the ER negative patients responded. Tamoxifen appears to be a safe, effective agent in the treatment of metastatic breast cancer and the response rate correlates highly to the presence of estrogen receptor protein. (Author abstract) (no Refs)

37. CORRELATION OF ESTROGEN RECEPTOR SITES TO RESPONSE: REPORT OF 11 PATIENTS WITH BREAST CANCER TREATED WITH TAMOXIFEN.

Lerner H, Band P, Israel L, Leung B
Pennsylvania Hospital, Philadelphia, PA 19107
Proc Am Assoc Cancer Res; 17:2 1976

Breast cancer specimens were taken in the operating room of the Pennsylvania Hospital from 11 patients. The tumor was immediately frozen in liquid nitrogen, and estrogen receptor (ER) analyses were performed by Dr. B. Leung using the dextran coated charcoal assay. Tumors having estrogen binding sites greater than 1 femtomole/mg of protein are reported as ER positive. All of the patients in this series had measurable disease and were treated with tamoxifen, 10 mgs., bid. Four patients were ER negative; none of these responded. 7 patients were ER positive; of these, there were 4 complete responses and 2 partial responses. All responses in this series were in soft tissue. The most common side effect was "hot flashes." This was only noted in the responders. (Author Abstract)

38. THERAPEUTIC USE OF TAMOXIFEN IN ADVANCED BREAST CANCER: A CORRELATION WITH BIOCHEMICAL PARAMETERS (MEETING ABSTRACT).

Morgan LR, Schein PS, Hoth D, McDonald J, Posey LE, Beazley RW, Trench L
Depts Pharmacol, Med, Surg and Pathol, La St Univ Med Ctr, New Orleans, La 70112, Vincent T Lombardi Cancer Res Ctr, Washington, DC 20007 and Ici Us, Wilmington, Del 19897
Proc Am Assoc Cancer Res; 17: 26 1976

Previous studies have shown that patients whose tumors have estrogen-binding proteins (EBP) and elevated arylsulfatase B (AS-B) activities responded favorably to endocrine ablation. In this study, Tamoxifen, a competitor for EBP, was investigated for its efficacy in tumor palliation. Sixty-three patients were evaluated for their responses to Tamoxifen given 20 mg daily. Objective responses were noted in 21/63 (33%) patients; 9/21 (43%) responders were greater than 10 years postmenopausal. The CR/PR/NR ratios were: premenopausal - 0/3/7 (30%); post-ablation - 0/1/2 (33%); postmenopausal - 1/16/33 (34%). Of 24 patients whose tumor biopsies contained less than EBP, 1 experienced CR, 5 PR and 18 progressed on Tamoxifen therapy. AS-B activities agreed with EBP values. Low EBP and low AS-B values were detected in lesions from 8 other patients; all of whom progressed on therapy. Patients greater than 5 years postmenopausal responded more often and 16/21 responders had only received cobalt as prior therapy. Side effects were bone pain at sites of tumor, nausea/vomiting, skin reaction and thrombocytopenia. (Author Abstract)

39. THERAPEUTIC USE OF TAMOXIFEN IN ADVANCED BREAST CANCER: CORRELATION WITH BIOCHEMICAL PARAMETERS.

Morgan LR, Schein PS, Woolley PV, Hoth D, MacDonald J, Lippman M, Posey LE, Beazley RW
Department of Pharmacology, Louisiana State University Medical Center, 1542 Tulane Ave, New Orleans, La 70112.

Cancer Treat Rep; 60(10):1437-1443 1976

Tamoxifen (NSC-180973; ICI-46474) can provide palliation to patients with advanced breast cancer whose tumors contain estrogen-binding proteins (EBP). The drug is most effective in patients with bone metastasis and minimal prior therapy. In the present study, 72 patients with advanced breast cancer were evaluated for their response to oral tamoxifen therapy administered at a dose of 20 mg twice daily. Twenty-eight of 72 patients (38%) demonstrated objective responses to tamoxifen therapy. For patients with a positive EBP and no prior chemotherapy, eight of 11 (74%) responded. No patient possessing a tumor negative for EBP achieved a remission. Patients with tumors that possessed normal arylsulfatase B and glucose-6-phosphate dehydrogenase enzyme activities responded most favorably to tamoxifen therapy. These results demonstrate that tamoxifen is effective in the treatment of patients with advanced breast cancer, and EBP and specific enzymes might be useful in selecting the patients for hormone manipulation. (Author Abstract)

40. PREDICTIVE CLASSIFICATION OF HUMAN BREAST CARCINOMAS BASED ON LACTALBUMIN SYNTHESIS.

Woods KL, Cove DH, Howell A
Dept. Medicine, Queen Elizabeth Hosp. Birmingham B15 2TH, England

Lancet; 2(8027):14-16 1977

The milk protein lactalbumin detected in the cytosol fraction of 20/53 breast carcinomas was assayed and correlated with the presence of cytoplasmic estrogen receptor. All but one of the tumors containing lactalbumin also had demonstrable estrogen receptor. Estrogen receptor was found in 36 of the tumors and lactalbumin was detected in 19 of these. The highest estrogen receptor concentrations were found in five tumors that did not contain measurable lactalbumin. The presence of lactalbumin was closely associated with the presence of estrogen receptor. Synthesis of lactalbumin appears to be conditioned upon a functioning estrogen receptor mechanism. (11 Refs)

41. ESTROGEN RECEPTOR ASSAY IN THE DIFFERENTIAL DIAGNOSIS OF ADENOCARCINOMAS.

Kiang DT, Kennedy BJ
Box 168, Univ. Hosp., Minneapolis, MN 55455

JAMA; 238(1):32-34 1977

Tumor tissue from various origins and from 295 patients was used in an evaluation of estrogen receptor determination as a means of locating the type and primary site of the carcinomas. Estrogen receptor was determined by differential sucrose gradient sedimentation and it was shown that the estrogen receptor sediments at 8S. Estrogen receptor is a cytosol protein found in estrogenic target tissues and mammary tumors. Tumor tissue from 295 patients with malignant disease of known primary site was examined using the estrogen receptor determination and it was observed that 8S type of receptor was found only in patients with adenocarcinoma of the breast, ovary, and uterus. Estrogen receptor determination was found to be useful in determining the primary site in 5/19 patients with carcinoma of unknown primary involvement. The assay was also useful in differentiating bilateral primary from metastatic breast cancer in two additional cases. Clinical histories of five of these seven patients are presented. It is concluded that information distinguishing new primary sites from metastases can be obtained when the two tumor specimens differ noticeably in their estrogen receptor content. (16 Refs)

42. CHARACTERISTICS OF THE ESTRADIOL RECEPTOR (E2R) ASSAY IN BREAST TUMOR PREPARATIONS.

Shafie S, Brooks SC
Dept. of Biochem., Wayne State Univ. School of Medicine, Detroit, Mich. 48201

Proc Am Assoc Cancer Res; 17:124 1976

The widespread use of various modifications of the dextran-coated charcoal assay (DCC) for the quantitation of E2R in human breast tumors makes it imperative that the parameters of the assay be standardized. Utilizing 10' g supernatant from breast tumor biopsies (HBT) or the breast tumor cell line MCF-7, reducing agents increased the apparent E2R in the order: dithiothreitol (DTT) greater than glutathione greater than a-monothioglycerol. When added to MCF-7 cytosol, the optimum DTT level (15mM) increased E2R to 2X control (no DTT). In HBT "tissue-sol" the optimum DTT level (lmM) increased E2R 1.tX. E2R was decreased as much as 70% of control when reducing agents were added in concentrations above or below optimum levels. Maximum competition by unlabeled E2 with ^{3}H-E2 for E2R required 200X excess E2 in HBT "tissue-sol" and 500X E2 in MCF-7 cytosol. The magnitude of competitive binding for E2R was in the order E2 greater than DES greater than U11, 100A greater than estrone. There was a 9% loss of E2R per mg DCC (1:10) when the total protein per sample was below 60ug. Supplementation with 200.mu.g albumin prevented this loss. The dextran:charcoal ratio also prevented E2R loss in the order: 1:1 greater than 1:10 greater than 1:100. One mg of DCC (1:10) has the capacity to absorb 0.3-0.4 g of E2. Other competitors adsorbed to DCC (1:10) in the order: DES greater than U11,100A greater than E2 greater than E1. These data provide a quantitative evaluation of the factors which influence the measurement of E2R. (Author Abstract)

43. TUMOR ESTROGEN RECEPTOR, SERUM PROTEIN ELECTROPHORESIS AND IMMUNOGLOBULIN LEVELS IN BREAST CARCINOMA (MEETING ABSTRACT).

Markland FS, Lee YN, Chandor SB, Sakulsky B
University of Southern California School of Medicine, Los Angeles, Cal. 90033

Proc Am Soc Clin Oncol; 17:302 1976

Estrogen receptors (ER) were measured on biopsy specimen taken from 54 patients with breast cancer. Using sucrose gradient assay, 16 patients had ER- tumors, 17 ER plus or minus (1-7 fmole/mg protein), and 11 ER+. All patients also had CEA, serum protein electrophoresis, quantitative immunoglobulins, and delayed cutaneous hypersensitivity test to 7 recall skin test antigens. Preliminary analysis showed that ER levels did not correlate with age, peripheral lymphocyte count, concentrations of immunoglobulin G, A or M. But patients with high ER values had the lowest alpha-2 globulins. This is interesting, because we found that patients who were free of breast cancer had the highest albumin and the lowest alpha-2 globulin during follow-up examinations as compared to those who still have breast cancer, early or advanced (unpublished data). (Author Abstract)

44. CARCINOEMBRYONIC ANTIGEN (CEA) IN PATIENTS WITH BREAST CANCER.

Borthwick NM, Wilson DW, Bell PA
Tenovus Institute for Cancer Research, Welsh National School of Medicine, Heath, Cardiff, CF4 4XX, Wales, United Kingdom

Eur J Cancer; 13(2):171-176 1977

Plasma CEA concentrations were measured in normal women and in patients with both primary and advanced breast carcinoma by means of a radioimmunoassay utilising rabbit anti-CEA antiserum. Fifty-five per cent of women with primary breast cancer had detectable levels of CEA (greater than 0.1 U/ml), as compared to 13% in a random selection of the female population. The incidence of detectable CEA levels in benign conditions of the breast was less than 10%, but the largest incidence of detectable levels of CEA (70%) was in the group of patients with advanced breast carcinoma. In patients with primary cancer, no correlation was observed between the plasma CEA levels and either the presence of oestradiol-17 beta receptor proteins in the tumours or the menstrual status of the patient. The effect of anti-oestrogen therapy on plasma CEA concentration in patients with advanced breast cancer was studied. A greater proportion of patients classified as clinical non-responders showed increased plasma CEA concentrations and fewer showed decreased CEA levels than in the case of the patients who responded to treatment. (20 Refs). (Author Abstract)

45. INTERACTION OF O,P'DDT WITH THE ESTROGEN-BINDING PROTEIN (EBP) IN HUMAN MAMMARY AND UTERINE TUMORS.

Kupfer D, Bulger WH
Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Res Commun Chem Pathol Pharmacol; 16(3):451-462 1977

The in vitro effect of o,p'DDT on the binding of 'H-estradiol-17 beta ('H-E2) to the high affinity estrogen-binding protein (EBP) in cytosol derived from human mammary and uterine tumors was examined. o,p'DDT inhibited the binding of 'H-E2 to EBP in both breast and uterine tumors. Using Scatchard and Linweaver-Burk plot analyses, the inhibition by o,p'DDT of 'H-E2 binding to mammary tumors EBP appears to result from competition for the 8S moiety binding sites and not from deactivation of the binding sites. The K1 for the inhibition by o,p'DDT with E2 binding was determined, yielding a value of 3.5 plus or minus 0.1 muM. The possible relevance of these findings to human population exposed to DDT derivatives is discussed. (26 Refs). (Author Abstract)

46. THE PREDICTION OF HORMONAL DEPENDENCY OF MAMMARY CANCER.

Block GE, Jensen EV, Polley TZ
University of Chicago Hospitals and Clinics, 950 East 59th Street, Chicago, Illinois 60637

Ann Surg; 182(3):342-352 1975

The estrogen receptor protein (estrophilin) was determined in specimens from 359 primary breast cancers and from metastatic tumors of 214 patients. Ninety-eight patients were eventually treated by some form of endocrine therapy: 82 patients were treated by ablative therapy, and 16 by hormonal additive treatment. The records of 81 of the 98 patients whose tumors were characterized for estrogen receptor content and who had received some type of endocrine therapy were reviewed by a peer review group that assessed the objective data for these patients regarding objective remission or failure to treatment. A positive estrophilin determination was defined as receptor content greater than 250 fmole per gram of tumor for premenopausal females and greater that 750 fmole per gram of tumor for postmenopausal and previously castrated patients. Estrogen receptor determinations may be made from tumor specimens as small as 200 mg. Sixty-nine patients underwent some form of ablative therapy. Twenty-seven of these patients were found to have significant receptor determinations. Of those patients in whom a definite determination was made of their post-treatment response, roughly two-thirds experienced objective remissions. None of the 42 patients with negative estrogen receptor determination experienced an objective remission to ablative endocrine therapy. Only one of 6 patients with negative determinations benefited from additive hormonal therapy; 4 of 6 patients with positive determinations benefited from additive therapy. Not all women with receptor containing mammary cancer will respond favorably to endocrine therapy, but those patients whose tumors lack a critical amount of estrophilin have little chance of benefit from either endocrine ablation or hormone administration. The estrogen receptor content of the primary tumor indicates the hormonal dependency of the tumor and may be used to predict the response to endocrine treatment when recurrent disease appears. (Author Abstract)

47. EVALUATION OF ESTROGEN RECEPTOR ASSAYS IN HUMAN BREAST CANCER TISSUE.

McGuire WL, De La Garza M, Chamness GC
Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284

Cancer Res; 37(3):637-639 1977

Standard dextran-coated charcoal (DCC) and sucrose gradient centrifugation assays for estrogen receptor were compared in a series of human breast cancer tissues. From a quantitative standpoint the results were remarkably similar. A simplified version of the DCC assay compared to the sucrose gradient assay yielded acceptable results. We conclude that, in spite of the lack of specificity controls inherent in the sophisticated standard assays, the simplified DCC assay might be useful if the biopsy specimen is too small to provide the number of aliquots for a standard DCC assay or sufficient protein for a sucrose gradient analysis. It also might be useful in research laboratories attempting to develop assays for multiple receptors or other constituents in a single tumor biopsy specimen. (Author Abstract)

48. COMPARATIVE ESTROGENIC PROPERTIES OF ESTRONE MUSTARDS AND DERIVATIVES. (MEETING ABSTRACT).

Lui YH, Morgan LR, Hagardorn AN
Dept of Pharmacol La St Univ Med Ctr, New Orleans, La 70112

Fed Proc; 36(3):335 1977

The use of cytoplasm estrogen binding protein (EBP) to predict response to therapy in human breast cancer continues to be supported. In this study we have systematically compared the EBP binding capacities of ring A substituted estrones to their estrogenic (vag mat index) properties in vivo. A number of substituted estrones were compared: NO2-, NH2-, CH3-, N-alkyl amino-, and C1-CH2-CH2-N-. In all cases the 2-substituted estrones that could form hydrogen bonding with the 3-OH group had low to no binding capacity. Substitution of bulky groups, eg morpholino in the 2-position destroyed activity. The EBP binding capacities agreed with the in vivo estrogenic activities. Steric, electronic and hydrogen bonding factors were compared. Effects of substitution on the A ring of estrone and 17beta-estradiol are reflected in the absorption spectra. The spectroscopic data (energy level, extinction coefficient) can be correlated with the estrogenic activity and the binding capacity. Cytotoxic and estrogenic properties of 2- and 4-substituted estrone mustards (-Cl, -SO2CH3, -SO2tosyl) will be correlated with their EBP binding properties and antiestrogen activities in mouse mammary tumors. (Author Abstract)

49. MAMMARY CANCER. TISSUE ESTROGEN RECEPTOR ASSAY AT ALBANY MEDICAL CENTER.

Holt JA, Plummer JM, Swartz DP, Sponzo RW, Horton J, Jacobson HI

MS-162 Dept of Obstetrics and Gynecology Albany Medical Coll 47 New Scotland Ave, Albany, NY 12208

NY State J Med; 77(6):903-907 1977

The (TERA) tissue estrogen receptor assay in mammary tumor tissue provides the physician with the most important single indicator yet devised for assessing the likelihood that the breast cancer patient will benefit from endocrine therapy. The cytoplasmic estrogen receptor protein is labile and requires explicit guidelines in regard to specimen handling and processing. A properly chilled segment of the surgical specimen must be delivered to avert a false negative assay result. There is evidence that the estrogen-binding protein being measured is in fact a receptor; the parameters provided in evidence are the affinity constant for hormone binding as determined from Scatchard plots and the sedimentation velocity exhibited by the protein-hormone complex during ultracentrifugation on a sucrose gradient. The endocrine status of the patient must be considered in interpreting TERA results. We have analyzed 74 specimens obtained from patients in the Albany area. Of these, 31 were interpreted to indicate probable response to endocrine therapy. The distribution by endocrine status was as follows: Of 61 primary tumors analyzed, probable response was indicated in 2 of 24 premenopausal, 0 of 5 perimenopausal, and 21 of 42 postmenopausal patients. Of 13 recurrent breast tumor specimens analyzed, probable response was indicated in 3 of 3 premenopausal, 3 of 4 perimenopausal, and 2 of 6 postmenopausal patients. These data indicate no apparent difference between breast cancer patients of the Albany area and those of other regions with respect to TERA-aided prediction of response to endocrine therapy. Estrogen receptor was found in all 15 specimens of pathology-free uterine tissue obtained from premenopausal women, and none was detected in two thyroid tumors. (25 Refs) (Author Abstract)

50. ESTROGEN RECEPTORS IN ANDROGEN-INDUCED BREAST TUMOR REGRESSION.

Zava DT, McGuire WL

Dept of Medicine, Univ of Texas Health Science Center, San Antonio, TX 78284

Cancer Res; 37(6):1608-1610 1977

The hormone-dependent 7,12-dimethylbenz(a)anthracene rat mammary tumor has been shown to regress when administered pharmacological doses of testosterone propionate. Tumor regression was correlated with estrogen receptor before and 15 to 20 days following testosterone therapy. A dramatic decline of receptor occurred in all regressing tumors, whereas those administered sesame oil alone maintained both growth and receptor content. Although receptor in regressing tumors was significantly less than in the untreated biopsies, the small amount of remaining receptor maintained the same binding affinity to estradiol, showing that testosterone affects the number and not estrogen affinity of the estrogen receptor. These studies suggest that testosterone depletion of estrogen receptor may be causally related to tumor regression. (23 Refs) (Author Abstract)

51. STEROID RECEPTORS AND THE CONTROL OF MAMMARY TUMOR GROWTH. (PP. 155-163)

King RJ, Smith JA

Breast Cancer, Trends in Research and Treatment A Monograph of the European Organization for Research on Treatment of Cancer. New York, Raven Press, 1976.

Factors affecting proliferation of mammary tumor cells are discussed, and several ways in which a mammary tumor containing abundant cytoplasmic estrogen receptor (RE) might not respond to endocrine therapy are described. Steroids are only one of many factors regulating proliferation. For example, in S115 mouse mammary tumor cells, three characteristics relate to ways in which their proliferation might be regulated: they grow in the absence of steroid, their growth rate is approx doubled by androgen, and proliferation is density regulated. In the absence of steroid, receptor protein might directly affect the proliferative machinery, the addition of steroid increasing the efficiency of the receptor, or the steroid may act indirectly by making the cell more sensitive to other stimuli. Proliferation is also increased by serum, and other stimulatory factors include immunological factors, blood supply, and tumor encapsulation. Other potential causes of false-positive tumors include defects in the receptor mechanism (defective nuclear transfer of a cytoplasmic receptor as evidenced by the estrogen-induced hamster kidney tumor, spontaneous GRA mouse mammary tumor systems, and glucocorticoid-sensitive lymphoma tumor) and defects in the postreceptor sequence of events, with expression of the steroid effect blocked at a point distal to chromatin activation (as in some glucocorticoid-sensitive leukemias, androgen-responsive mouse mammary tumors and glucocorticoid-sensitive hepatoma cells). In human breast tumors, the inactive 4S RE is not distinguishable from other active receptors in current assays except sucrose gradient analysis. However, the clinical correlation of this receptor with unresponsiveness is not yet clear. Tumors with heterogeneous populations of cells could also behave as a false-positive. Limited clinical data indicate that RE measurements should indicate tumor response to a spectrum of treatments ranging from hypophysectomy to androgen therapy; the problem of prognosis for different types of therapy may be overcome by extending the range of hormone receptors measured. Defects in receptor mechanism beyond the cytoplasmic R can be resolved by better methodology, and the range of clinical tests should be as broad as possible so that many tumor-related factors can be measured. Other possible mechanisms noted in a discussion include defective genes, cellular metabolism, inadequate clinical trials, and mutation. (31 refs)

52. HORMONE-RESPONSIVE HUMAN BREAST CANCER IN CONTINUOUS TISSUE CULTURE. (PP. 111-139)

Lippman M

Breast Cancer, Trends in Research and Treatment A Monograph of the European Organization for Research on Treatment of Cancer Heuson JC, Mattheiem WH, Rozencweig M, ed. New York, Raven Press, 1976.

The establishment and characteristics of five human breast cancer lines in continuous tissue culture are reviewed. Each of the cell lines has varying responses to glucocorticoids, androgens, and estrogens; one cell line, MCF-7, showed marked responsiveness to estrogen, and the presence of a high-affinity estrogen receptor in these cells was confirmed. Many studies suggest than antiestrogens inhibit cells by competing with estrogen for specific receptor sites; the ability of increasing concentrations of tamoxifen to displace 10**-9 M 'H-estradiol from receptor was compared with the effect of increasing concentrations of tamoxifen on 'H-thymidine incorporation; these concentrations agree closely. The quantity of estradiol receptor in various cell lines as determined by dextran-coated charcoal assay was 71.6 femtomoles (fmoles)/mg protein for the MCF-7 cell line, 6.6 fmoles for MDA 231, 4.2 fmoles for HT-39, and 5.0 fmoles for G-11. Although estrogen clearly stimulates the cells above control levels, estrogen-deficient cells do grow; however, antiestrogen treatment is lethal, though estrogen reverses the effects and does not inhibit breast cancer cell lines that lack receptor. After 24 hr incubation in dexamethasone (10**-7 M), MCF-7

cell division is significantly slowed, but the cells eventually grow to confluence and do not appear to be killed by glucocorticoid. G-11, MCF-7, and MDA 231 have significant amounts of glucocorticoid receptor and show a hormonally provoked decrease in thymidine incorporation. Sucrose density gradient studies with MCF-7 suggest a receptor of limited capacity and some specificity for substances with androgenic or antiandrogenic properties. G-11, HT-39, and MDA 231 contain little receptor activity and are essentially unresponsive to androgen additions. These studies with estrogen-, glucocorticoid-, and androgen-responsive cell lines strongly support the notion that a lack of receptor is uniformly associated with absence of relevant responses to steroid, but the converse is not considered true. (16 refs)

53. STEROID RECEPTORS IN BREAST NEOPLASIA. (PP. 67-82)

DeSombre ER, Jensen EV

Control Mechanisms in Cancer. Criss WE, Ono TO, Sabine JR, ed. New York, Raven Press, Progress in Cancer Research and Therapy, vol. 1, 1976.

The main aspects of estrogen interaction with its cell receptor protein, estrophilin, in hormone-dependent tumors were reviewed and the clinical applications discussed. Estrogen binding was correlated with response to endocrine therapy in 133 women with advanced breast carcinoma. Estradiol binding capacity in 123 varied from 0 to 20 picomoles/g of tumor. No case without ovarian function whose tumor had less than 2.5 picomoles/g and no premenopausal patient with less than 1.0 picomole/g was responsive. These two values define the critical limits of positive response. Ablative therapy was undertaken in 104/133 patients. Clinical remissions were categorized as positive, borderline, or negative in receptor test results and were, respectively, 2/4, 0/8, 0/11 for adrenalectomy, 13/18, 0/2, 1/15 for adrenalectomy plus oophorectomy, 2/4, 0/1, 0/8 for hypophysectomy, and 5/7, 0/0, 1/27 for oophorectomy. In 29 patients with hormone therapy, the clinical remissions per test result were 0/1, 0/0, 0/3 for androgen, 2/2, 0/2, 1/6 for estrogen, 1/2, 0/0, 0/1 for antiestrogen, and 3/5, 0/0, 0/7 for estrogen plus progestin. Among the positive responders there were 28/43 remissions and 3/90 in the borderline or negative cases. In general, about 2/3 of the patients with significant estrophilin levels will benefit from hormone management. In another group of 800 women with advanced primary or metastatic breast carcinoma, 30% were receptor-rich and 70% were receptor-poor. Estrophilin assay can forecast an optimal therapy in about 90% of all patients. Simultaneous assay of both estrogen receptors (ER) and progesterone receptors (PR) (with tritiated H-R5020) found the highest concentrations of PR in tumors rich in ER; most of the estrogen-poor receptors do not have PR. Estrophilin levels of primary tumor, assayed at mastectomy, can forecast responses to endocrine therapy during the development of later metastases. Clinical remission with endocrine therapy 1-2 yr later was found in two patients with receptor-rich primary tumors; 10/11 patients with receptor-poor primary tumors failed to respond to endocrine therapy for metastatic control 4 mo-5 yr later. (62 refs)

54. HORMONE RECEPTORS AND ENDOCRINE THERAPY IN HUMAN BREAST CANCER. (PP. 39-44)

McGuire WL, Horwitz KB

Hormones and Breast Cancer Symposium organise par le Centre Antoine Lacassagne in Nice, 23-24 May 1975. INSERM Nice France, vol. 55, 1975.

The clinical correlations of estrogen receptor (ER)-content of tumor and the response to endocrine therapy in 436 trials on 380 patients with advanced breast cancer are reported. Tumors with values greater than 3mg/mg cytosol protein were considered positive (ER+). Of 107 patients with ER+ tumors, 59 had an objective response to ablative therapy, compared to 8/94 with ER- tumors and 3/10 with borderline ER content. Response to androgen, estrogen, or glucocorticoid therapy occurred in 51/85 with ER+ tumors, 7/82 with ER- tumors, and 0/3 of those with borderline tumors. The response rate to anti-estrogens and other endocrine therapies was 10/23 in patients with ER+ tumors and 5/32 in those with ER- tumors. ER determination can, thus, be used to spare a large number of patients (ER-) from unrewarding major endocrine ablative procedures. Progesterone receptors (PgR) were determined in over 100 human mammary tumors. PgR was absent in all of the tumors lacking ER, and 56% of the ER+ tumors had PgR. The findings are consistent with the hypotheses that PgR can be used as a marker for hormone dependence. It is suggested that if these results are confirmed, the 40% of ER+ tumors which will be endocrine resistant will be identifiable. (18 refs)

55. MEASUREMENT OF ESTRADIOL RECEPTORS IN HUMAN BREAST TUMORS BY POLYACRYLAMIDE GEL ELECTROPHORESIS.

Duffy MJ, Duffy GJ

Dept. Nuclear Med., St. Vincent's Hosp., Dublin 4, Ireland

Clin Chim Acta; 74(2):161-166 1977

Specific estradiol receptors in cytosols from human breast tumors were measured by discontinuous polyacrylamide gel electrophoresis. Binding of estradiol to contaminating sex-hormone binding globulin was clearly separated from that due to the specific receptor. The amount of estradiol bound by the receptor was linear with respect to the amount of protein added to gel. The estradiol receptor peak of radioactivity showed saturability (at 5 x 10**-8 M) and specificity characteristic of an intracellular receptor. Of the naturally occurring nonlabeled steroids examined, only estradiol and estriol significantly decreased the amount of radioactive estradiol bound. Testerone, progesterone, and cortisol had no significant effect. The results show that polyacrylamide gel electrophoresis can be used to quantitate specific estradiol receptors in the presence of other high-affinity binding components. (21 refs)

56. THE EFFECTS OF ANDROGENS AND ANTIANDROGENS ON HORMONE-RESPONSIVE HUMAN BREAST CANCER IN LONG-TERM TISSUE CULTURE.

Lippman M, Bolan G, Huff K

Medicine Branch, NCI, Bethesda, MD 20014

Cancer Res; 36(12):4610-4618 1976

The effects of various steroids on cell growth and on precursor incorporation by a human breast cancer line, MCF-7, are reported. At 10**-7 M, 5a-dihydrotestosterone (5a-DHT) stimulated cell division after 2 days in culture, but at the same concentration, the antiandrogen R2956 (17b-hydroxy-2,2,17a- trimethoxyestra-4,9,11-triene-1-one) was inhibitory and exposure for more than 72 hr was lethal. Amino acid and nucleoside incorporation began to be stimulated at 5a-DHT concentrations over 10**-9 M, and at 10**-6 M there was a 300% increase in thymidine incorporation and a 200% increase in leucine incorporation. 5a-DHT was less effective at concentrations above 10**-6 M. The stereoisomer 5b-DHT was not stimulatory at any concentration examined. A high-affinity receptor site was demonstrated in cytosols by sucrose density gradients and by competitive protein-binding analysis, and 45 femtomoles of ^{3}H-DHT were bound per milligram of cytoplasmic protein. Cross-competition studies revealed the presence of androgen and estrogen receptors that were distinguishable from each other. The androgen receptor had a much lower binding affinity for estrogen than for DHT, and the estrogen receptor had virtually no affinity for estrogens.

17b-estradiol and the antiandrogen cyproterone acetate appeared to have similar affinities for the androgen receptor. Binding studies indicated that testosterone was bound to a single class of receptors of uniform affinity. The dissociation constant for testosterone was 1.3 x 10**-9 M, approx twice that obtained for 5a-DHT. There was an approx 100-to 1,000-fold discrepancy between concentrations of DHT that half-maximally saturated the androgen receptor and concentrations that half-maximally stimulated either leucine or thymidine incorporation, the latter being the higher. Changes in quantity and affinity of androgen binding to intact cells at 37 C compared with the usual binding techniques at 0 C with cytosols did not explain the discrepancy. Incubation of 5a-DHT with cell suspensions resulted in conversion to a steroid that comigrated with 5a-androstanediol chromatographically, the conversion being approx 70% complete after 8 hr. Examination of incubation media, cytoplasmic extracts, and crude nuclear pellets indicated the probable conversion of ^{3}H-testosterone to ^{3}H-5a-dihydrotestosterone. Cyproterone acetate by itself had little effect on thymidine incorporation at concentrations under 10**-6 M, but some inhibition was apparent at 10**-6 M. At 10**-5 M, cyproterone acetate significantly decreased the stimulatory effect of 10**-6 M 5a-DHT. The in vitro androgen-dependence of this line of human breast cancer is emphasized. (22 refs)

57. FREEZING AND STORAGE OF BREAST CANCER TISSUE FOR ESTROGEN RECEPTOR PROTEIN ASSAY. A CONVENIENT METHOD.

Brown PW, Witorsch RJ, Banks WL, Lawrence W
Div. Surgical Oncology, Box 11, Medical Coll. Virginia Hosp., Richmond, VA 23298
Arch Surg; 112(2):183-185 1977

Freezing and storage of breast cancer tissue for estrogen receptor protein assay was investigated in biopsies obtained from 16 patients who underwent biopsy of primary or metastatic breast cancer. The tumors were frozen by either liquid nitrogen or the fluorinated hydrocarbon spray, Cryowik. The estrogen receptor protein (ERP) assay was performed and similar results were obtained with both specimens. Three patients had ERP measured after approx 14 wk storage: there was no statistically significant loss of activity by either method of preparation. The Cryowik preparation method makes possible the accurate measurement of ERP in all community hospitals. (4 refs)

58. ISOELECTRIC FOCUSING OF ESTRADIOL RECEPTOR PROTEIN FROM HUMAN MAMMARY CARCINOMA - A COMPARISON TO SUCROSE GRADIENT ANALYSIS.

Wrange O, Nordenskjold B, Silfversward C, Granberg PO, Gustafsson J
Dept. Chemistry, Karolinska Institutet, S-104 01 Stockholm, 60, Sweden
Eur J Cancer; 12(9):695-700 1976

Tissue from 26 human mammary tumors was subjected to two methods of analysis for estrogen receptor protein: sucrose gradient analysis (SG) and isoelectric focusing analysis (IF). Nine of the 14 SG-positive tumors demonstrated a single 4S complex, and the other 5 had both a 4S and an 8S complex. The 8S complex was always saturable with an excess of unlabeled estradiol, while the 4S complex contained variable amounts of nonsaturable binding sites. IF analysis yielded 12 positive specimens with receptor protein focusing at approx pH 6.6. Sometimes a double peak appeared with a max at pH 6.65. The pI 6.6 (isoelectric point at pH 6.6) complex was always saturable with unlabeled estradiol. There was one IF-positive GS-negative tumor, and three specimens were SG-positive and IF-negative. Six IF-positive specimens showed only 4S peaks in sucrose gradients, and all six tumors with both 4S and 8S complexes were IF-positive. It is concluded that there is a positive correlation between SG analysis and IF analysis, and that this correlation is stronger when SG analysis demonstrated both 4S and 8S complexes. There was an increased frequency of receptor positive tumors among differentiated carcinomas. Preliminary studies have indicated that the 4S focused at pI 6.6 and that the 8S form precipitated under the conditions used. Final evaluation of IF for measurement of estradiol receptors in mammary carcinoma must await correlation with results of endocrine therapy. (10 refs)

59. EFFECT OF CA^{2}+ AND SALT ON FORMS OF ESTRADIOL CYTOPLASMIC RECEPTOR IN HUMAN NEOPLASTIC BREAST TISSUE.

Schneider SL, Dao TL
Dept. Breast Surgery and Breast Cancer Res. Unit, Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY 14263
Cancer Res; 37(2):382-387 1977

After incubation with ^{3}Hestradiol, low-salt sucrose gradient centrifugation of 250 human breast tumor cytosols revealed receptor-bound steroid in both 4S and 8S forms. Cytosols prepared from 24 of these receptor-positive tumors were examined in the presence of KCl, Ca^{2}+, and the serine protease inhibitor, phenylmethylsulfonyl fluoride. After incubation with KCl (0.4 M), interconversion of 8S and 4S forms was found in half the 24 cytosols, the original low-salt patterns of which contained well-defined 4S and 8S peaks. When cytosols were made 4 millimolar in Ca^{2}+ 15-30 min before the addition of KCl, an additional 4.5 S form was observed in low-salt gradients in 18 tumors; inactivation of receptor occurred in 6 tumors. The effects did not occur when Ca^{2}+ and salt were added simultaneously. After 2-24 hr of preincubation of the same cytosols with Ca^{2}+, under the same conditions, recovery of the 4S receptor complex increased in low-salt gradient. These Ca^{2}+ effects were inhibited if phenylmethylsulfonyl fluoride and Ca^{2}+ were added simultaneously to cytosols prior to the addition of salt. Human mammary tumor cytosols may contain proteolytic effects, which can be activated by Ca^{2}+ and which can produce irreversible changes in the salt-dissociated steroid receptor proteins, which can be detected in sucrose density gradients. (27 refs)

60. PROGESTERONE AND ESTRADIOL RECEPTORS IN HUMAN BREAST CANCER (MEETING ABSTRACT).

Huber PR, Geyer E, Wyss HI, Eppenberger U
Hormonlabor, Universitatesfrauenklinik, Schanzenstrasse 46, CH-4004 Basel, Switzerland
Experientia; 32(6):798 1976

Sera of 200 breast tumor patients have been screened for alterations in the level of Estradiol (E2), Progesterone (P), Prolactin and Growth hormone. Our results as well as those of other workers have shown the plasma levels of above hormones to be normal in breast cancer patients. Therefore the reason for hormone dependency of breast carcinoma growth has to be looked for within the carcinoma cell itself. In our experiments we studied the content of estradiol receptors in 29 cases of human breast tumors and found that 40% contained the E2-binding protein. The receptor content was measured in 14 cases and 70% contained progesterone receptor. The tumor tissue which bind E2 and P had binding constants (Kd) of 0.04 nM and 0.03 nM respectively. In certain cases sucrose gradient studies carried out showed 4S and 8S binding for estradiol, which was completely abolished by U-11100 (an antiestrogen) indicating the specificity of E2-binding to both forms of the receptor. Similar experiments are

presently carried out on the progesterone receptor using the highly specific progestin R5020 (Roussel-Uclaf). These experiments indicate that progesterone is bound to a component which is different to the estradiol receptor, but has similar properties. These findings suggest that not only E2 and its receptor have to be taken in account in hormonal therapy of human breast cancer but the progesterone receptor content as well.

61. BREAST CANCER IN YOUNG WOMEN.

Diekamp U, Bitran J, Ferguson DJ

Dept. Medicine, Section Hematology/Oncology, Univ. Chicago, Chicago, IL 60637

J Reprod Med; 17(5):255-278 1976

The literature on diagnosis and treatment of breast cancer in women less than 40 yr old is reviewed. About 10% of female breast cancer patients are less than 40 and 10% of these are pregnant or nursing at the time of diagnosis. About 75% of breast cancers are discovered by the patient; however, by that time, the disease is relatively advanced. Immediate diagnostic and therapeutic action is essential for all cases. Complete removal of local and regional disease is the treatment of choice for all Stage I and II disease; limited surgery plus irradiation has not shown to be equal to radical surgery. Adjuvant cyclic combination chemotherapy may reduce recurrence and mortality rates. Determination of hormone receptor protein content in tumor tissue, at the time of surgery and recurrence, has been the most helpful indicator of hormone-dependent breast cancers. Adrenalectomy or hypophysectomy can benefit only 8% of all receptor-negative patients. During pregnancy, receptor studies require special techniques and simultaneous hormone level determinations. Combination chemotherapy with vincristine and prednisone, or cyclophosphamide, plus adriamycin have given 40-70% response rates in treating metastatic disease. The prognosis for young or pregnant breast cancer patients is the same for those in other age groups with the same extent of disease, but younger patients usually present with less extensive disease. (215 refs)

62. STEROID-BINDING PROTEINS IN CARCINOMA OF THE HUMAN MALE BREAST.

Thompson EB, Perlin E, Tormey D

Natl. Naval Medical Center, NCI, Bethesda, MD 20014

Am J Clin Pathol; 65(3):360-363 1976

A case of biopsy-proven infiltrating ductal carcinoma of the right breast in a 49-yr-old man is briefly reported. Steroid-binding proteins were studied in a biopsy specimen from the affected breast. The patient presented with an ulcerated breast, a palpable axillary node, and widespread bony metastases. He was treated with orchiectomy and local irradiation for bone pain, with resultant decrease of the primary lesion by approx 25% and moderate temporary relief of pain in the irradiated sites. Complete resolution of the primary and of the axillary node occurred on combination chemotherapy with vincristine, 5-fluorouracil, methotrexate, cyclophosphamide, and prednisone. Although lytic osseous lesions on x-ray did not completely resolve, there was complete disappearance of bone pain. Analysis of the biopsy specimen for steroid-binding proteins revealed significant binding sites for 17b estradiol (30 femtomole(fmole)/mg) and dihydroxytestosterone (50 fmol/mg), but not for testosterone (4 fmol/mg). The result for progesterone-binding (20 fmol/mg) was of uncertain significance, since contamination by other serum steroid-binding globulins could not be ruled out. It is concluded that some male breast carcinomas are under the influence of steroid hormones. (15 refs)

63. OESTROGEN RECEPTORS IN MALE BREAST CANCER.

Leclercq G, Verhest A, Deboel MC, Von Schoubroeck F, Mattheiem WH, Heuson JC

Service de Medecine et Laboratoire d'Investigation Clinique, 1 Rue Heger Bordet, Brussels, Belgium

Biomedicine Express; 25(9):327-330 1976

Estrogen receptors in 11 tissue samples from 7 primary and 4 metastatic male breast cancers were assayed by measuring the binding affinity of the cytosol fraction for H3-estradiol-17beta (H3-E2). The results indicate high-affinity binding sites for H3-E2 in 6/7 primary cancers and 1/4 metastases. In the positive lesions, the concentration of binding sites varied from 59 to 532 femtomoles/mg tissue proteins. When estrogen binding proteins from male and female breast cancers were compared for their specificity to bind E2, it was found that in each cytosol, E2 estrone, and an antiestrogen (U 11, 100 A) produced significant inhibition of the binding of H3-E2, whereas progesterone, testosterone, dihydrotestosterone, and androsterone, cortisone, and hydrocortisone were ineffective. No relationship was observed between amounts of estrogen binding proteins and patient age (46-72 yr). Chromosomal analysis showed that all patients had the 46 XX pattern without any autosomal or gonosomal anomalies. These results indicate that the use of estrogen receptors in male breast cancer as a guide to endocrine therapy may be subject to the same considerations as breast cancer in women. (17 refs)

64. ESTROGEN RECEPTOR PROTEIN IN LESIONS OF THE MALE BREAST: A PRELIMINARY REPORT.

Rosen PP, Menendez-Botet CJ, Nisselbaum JS, Schwartz MK, Urban JA

Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021

Cancer; 37(4):1866-1868 1976

Eleven specimens of breast lesions obtained from 10 male patients were analyzed for estrogen receptor protein (ERP). Three patients (ages 49, 77, and 82 yr) had infiltrating duct carcinomas with no axillary metastases. ERP in each of these was positive. Eight specimens with gynecomastia, one of which was obtained from the 77-yr-old patient with carcinoma in the same breast, were also analyzed. Of these, ERP was positive in a 59-yr-old man who had cirrhosis of the liver; two patients with borderline ERP had hepatitis and testicular seminoma, respectively. No relationship between histopathologic features of the lesions and ERP results was found. Review of available literature, including these cases, revealed that six of eight male breast carcinomas were ERP-positive.

65. ADVANCES IN BREAST CANCER RESEARCH. (PP. 31-49)

Dao TL

Perspectives in Cancer Research and Treatment Contributions in Honor of the Seventy-Fifth Anniversary of the Roswell Park Memorial Institute. Murphy GP, Pressman D, Mirand EA, ed. New York, Alan R Liss Inc, 1973.

Research that relates to the possibility of a hormonal approach to breast cancer therapy is reviewed. Experiments have demonstrated an interaction among prolactin, estrogen, and progesterone in regulating the growth of mammary cancer cells; one conclusion was that the stimulating effect of prolactin is estrogen-dependent. Two possible mechanisms by which estrogen regulates the effect of prolactin on tumor growth are proposed: prolactin is unable to initiate or maintain mitogenic activity of the cells, and ovarian hormones are needed to initiate DNA synthesis in the cells; ovarian hormones are known to increase the vascularity of tissues, so ovariectomy

may reduce the supply of prolactin to the mammary tumors by decreasing its access. The biochemical characteristics of hormone-dependent tumors, and specific estrogen binding proteins in target tissues and the sulfation of steroid hormones in mammary tumor tissues are discussed. Evidence that hormone-dependent breast cancer may become autonomously paraendocrine and begin synthesizing estrogenic hormones is presented. The prospect of lowering breast cancer mortality by hormone therapy depends partly on the careful selection of candidates, and it will be necessary to separate those patients with metastases from those in the early stages of the disease. Measurement of both estrophile protein and steroid conjugation enzymes in the primary tumor may be valuable in determining the course of the disease and the endocrine dependency of the tumor. (41 refs)

66. MECHANISMS OF ACTION OF ESTROGENS.

Hilf R, Wittliff JL
No affiliation given
Handbuch fuer Experimentelle Pharmakologie: Antineoplastic and Immunosuppressive Agents; (Springer, New York) 38(2):104-138 1974-1975

The current known mechanisms of action of estrogens are reviewed along with discussion of their chemical properties and therapeutic uses. The naturally occurring estrogens are estrone, estriol, and estradiol; they are steroids which contain 18 carbons. In vitro studies have shown that estrone and estradiol-17b are produced from C^{14} -labelled acetate in the ovaries and in the testes of humans. Tissues such as the placenta, ovaries, testes, and adrenal cortex can convert androstenedione and testosterone to estrogens. Estrogens markedly influence the levels of pituitary hormones, and low doses of estrogens increase plasma levels of luteinizing hormone, while larger doses will cause the opposite effect. Estrogens can increase the sensitivity of the ovary to respond to gonadotropins. Large doses of estrogen will produce a significant decrease in cholesterol concn in serum and adrenal glands of the rats. In pt with breast cancer, removal of estrogens by oophorectomy and/or adrenalectomy results in a significant temporary remission of cancer. Admin of estrogen to postmenopausal women with disseminated breast cancer results in objective remission in about 30% of pt. Studies of specific estrogen binding proteins in breast carcinomas and correlation with the clinical disease course provides another approach to identifying hormone-dependent neoplasia. Estrogens produce remissions in disseminated prostatic cancer, and this may be due to direct antiandrogenic action or to the ability of estrogens to inhibit pituitary gonadotropins. The uptake and retention of estradiol-17B by the uterus is an early step in the uterotrophic response, which precedes any alteration in rates of biosynthesis reactions known to be inhibited by actinomycin D or puromycin. Glycogen content in human endometrium varies at different stages of the menstrual cycle, and carbohydrate metabolisms may be important in providing energy for the actions of estrogens. (371 refs)

67. HORMONES AND NEOPLASIA.

Forrest AP
Dept. Surg., U. Edinburgh, Scotland
J Clin Pathol Suppl; 27(7):65-71 1974

The author presents a review limited to the hormonal influence on breast cancer. In about 1/3 of cancer cases, generally short-lived remissions are achieved by the administration of androgens or estrogens or the removal of the adrenals or pituitary. Normal development of the human breast requires complex hormonal influences, with estrogen, progesterone, prolactin, growth hormone, and cortisol all playing a part. Hormones can act as promoting agents in neoplastic transformation when given in unphysiologically large doses. Estrogen, progesterone, pituitary hormones, and prolactin all appear to influence the development of spontaneous mammary tumors in female mice and rats. Ovarian hormones also influence the behavior of human breast cancer, and there is evidence for the involvement of the pituitary. In addition, a strong relationship has emerged between the levels of the metabolites of the C-19 (androgenic) steroids in the urine and human breast cancer. The absolute levels of circulating hormones may be less important than the ability of the tumor to synthesize growth-promoting steroids which are locally active. Recent data has indicated a possible role of the sulfating enzymes for estrogens and a high affinity binding protein in the cytoplasm of human breast cancer in the development of breast tumors.

68. SPECIFIC BINDING PROTEIN FOR 17-B-ESTRADIOL IN PROSTATE WITH ADENOCARCINOMA.

Bashirelahi N, Young JD
666 W. Baltimore St., Baltimore, MD 21201
Urology; 8(6):553-558 1976

The presence of a specific estradiol-binding protein in prostates of patients with prostatic adenocarcinoma who were not previously treated with hormones is reported. Sucrose density gradient centrifugation patterns of 17 b-estradiol binding in cytosol obtained from a patient with prostatic adenocarcinoma are shown; cytosol was incubated alone with labeled or in the presence of unlabeled estradiol (1 x 10**-8 M or 1 x 10**-6 M, respectively), or alone with labeled estradiol (1 x 10**-8 M) plus unlabeled dihydrotestosterone (DHT: 1 x 10**-6 M) or in the presence of unlabeled estradiol (1 x 10**-6 M). Sedimentation patterns indicated the presence of binding protein(s) with a sedimentation coefficient of approx 3.6S. No specific DHT-binding protein was detected in sucrose density gradient centrifugation patterns of DHT binding in cytosol incubated alone with labeled DHT (1 x 10**-8 M) or in the presence of unlabeled DHT (1 x 10**-6 M). In a patient with chronic prostatitis and prostatic hyperplasia with no sign of adenocarcinoma, there was no specific estradiol-binding protein, but there was a distinct DHT-binding protein, as determined by sucrose density gradient centrifugation. In seven other patients with adenocarcinomas, specific estradiol binding was demonstrated in all prostates by means of the dextran-coated charcoal technique; DHT-binding was shown in three, and negligible amounts of DHT-binding protein were found in three. Thus, methods similar to those used in diagnosis and treatment of mammary cancer (correlating presence of estradiol receptors and success of endocrine therapy) might be used for classifying prostatic tumors. It is suggested that the method of choice of treatment of patients showing both estrogen and androgen receptors in their prostates would be a drug combination of estrogen and antiandrogen. (18 refs)

B. The Estrogen Receptor in Experimental Mammary Tumors

69. OESTROGEN BINDING AS A PREDICTIVE TEST FOR DMBA-INDUCED TUMOUR RESPONSE TO TAMOXIFEN THERAPY. (PP. 89-94)

Jordan VC, Jaspan T
Cancer Chemotherapy I. Hellmann K, Connors TA, ed. New York, Plenum Press, Chemotherapy, vol. 7, 1976.

The use of estrogen binding as a predictive test for 7,12-dimethylbenz(a) anthracene (DMBA)-induced tumor response to tamoxifen therapy was investigated. Tumor biopsies for estrogen binding studies were obtained 100 days after DMBA

administration (20 mg intragastric) to 50-day-old female Sprague Dawley rats. Each biopsied rat was immediately treated with 50 ug tamoxifen daily for 3 wk. Three types of tumor response to tamoxifen therapy were observed based on the estrogen binding of biopsy samples. In tumors with low estrogen binding (less than 11 femto-mole/mg cytosol protein), there was either stasis or tumor growth. At intermediate levels of estrogen binding (11-36 femto-mole/mg cytosol protein), an increased estrogen binding concentration was related to an increased tumor regression. However, at high levels of estrogen binding (greater than 36 femto-mole/mg cytosol protein), the expected 100% regression was not attained and often less than 50% regression was observed. The reason for the partial response of the last group remains obscure, although a larger daily dose of tamoxifen may have been more effective. Nevertheless, the practicality of using an estrogen receptor assay as a predictive test for DMBAinduced tumor response to tamoxifen therapy was demonstrated, suggesting that a similar approach in the treatment of human breast cancer may be useful. (17 refs)

70. EFFECTS OF OESTRADIOL-17.BETA. AND TAMOXIFEN ON TOTAL AND ACCESSIBLE CYTOPLASMIC OESTRADIOL - 17.BETA. RECEPTORS IN DMBA-INDUCED RAT MAMMARY TUMOURS.

Nicholson RI, Golder MP, Davies P, Griffiths K
Tenovus Institute for Cancer Research, Welsh National School of Medicine, Heath, Cardiff, CF4 4XX, Great Britain
Eur J Cancer; 12(9):711-717 1976

An exchange assay for the measurement of total cytoplasmic oestradiol-71 beta binding sites in DMBA-induced rat mammary tumours has been established. The assay was used to examine the in vivo interactions of tamoxifen and oestradiol-17 beta with cytoplasmic receptor proteins for oestradiol-17 beta. Although certain quantitative differences were observed between the actions of tamoxifen and oestradiol-17 beta, the compounds show an initial similarity of response, each ligand promoting an early elevation in receptor levels. Tamoxifen, however, was unable to sustain the replenishment of the cytoplasmic receptor. (Author Abstract)

71. EFFECTS OF OESTRADIOL-17BETA AND TAMOXIFEN ON NUCLEAR OESTRADIOL-17BETA RECEPTORS IN DMBA-INDUCED RAT MAMMARY TUMOURS.

Nicholson RI, Davies P, Griffiths K
Tenovus Institute for Cancer Research, Welsh National School of Medicine, Heath, Cardiff, CF4 4XX, Great Britain
Eur J Cancer; 13(3):201-208 1977

An exchange assay has been established for the measurement of nuclear oestradiol-17beta binding sites in DMBA-induced mammary tumours of the rat. The assay was used to examine the nuclear concentration of binding sites following the administration in vivo of tamoxifen and oestradiol-17beta. Both compounds translocate receptor protein to the nucleus and cause an early elevation in cytoplasmic receptor levels. By 24 and 48 hr after tamoxifen administration, the intranuclear concentration of oestrogen receptor complex has returned to levels indistinguishable from pretreatment values. At this time, replenishment of the cytoplasmic receptor had not occurred and tumours were non-responsive to oestradiol-17beta. (17 Refs). (Author Abstract)

72. KINETIC AND MOLECULAR PROPERTIES OF ESTROGEN BINDING PROTEINS IN LACTATING MAMMARY GLAND.

Boyd PA
University Rochester Cancer Center and Department Biochemistry, University Rochester Medical Center, Rochester, N.Y. 14642
Proc Am Assoc Cancer Res; 17:196 1976

Specific estrogen binding sites have been characterized using mammary glands of rats, 15-21 days postpartum. Rate constants of association (k1) of ^{3}H-estradiol-17 beta were 2-4 x 10^6 M**-1 min**-1 and those of dissociation (k-1) were 6-8 x 10**-3 min**-1 at 3 degrees C when analyzed by the dextran-coated charcoal procedure. Values of dissociation constants (Kd) obtained from Scatchard analyses and those calculated from kinetic data were comparable (2-5 x 10**-10M). Ligand specificity experiments using a competitive binding assay and various unlabeled steroids indicated that the functional groups at carbons 3 and 17 as well as unsaturation of the A ring influenced binding significantly. Binding sites were slightly more stable in the presence of estradiol (temperature inactivation coefficient, Ti=36 degrees C) than in its absence (Ti=30 degrees C) in cell-free preparation. However in tissue minces, binding capacity remained unchanged for 24 hr at 3 degrees C but decayed in a first order fashion at 25 degrees C with a half-life of 13 hr. Sedimentation properties as a function of ionic strength were defined by sucrose gradient centrifugation. These data provide a basis for comparison with estrogen binding proteins in mammary tumors. (Author Abstract)

73. HORMONE DEPENDENCE AND ESTRADIOL RECEPTORS IN THE D SERIES OF MAMMARY NODULE OUTGROWTH LINES AND TUMORS.

Medina D, Iramain CA, Clark JH
Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77025
Cancer Res; 35:2355-2360 1975

Preneoplastic mammary nodule outgrowth lines were examined for their ability to grow and produce tumors in ovariectomized BALB/c mice. In addition, these nodule outgrowth lines and tumors derived from them were investigated for cytoplasmic estrogen receptor proteins by qualitative and quantitative techniques. Early ovariectomy, performed within 4 weeks after transplantation, slightly delayed but did not permanently block the ability of three different nodule lines to completely fill the fat pad with nodule tissue. Ovariectomy performed later than 4 weeks after transplantation or adrenalectomy performed early or late had no effect on nodule growth. Neither early nor late ovariectomy or adrenalectomy had an effect on the maintenance of the nodule alveolar phenotype. Ovariectomy performed 3 weeks after transplantation had little effect on the tumor potential of the low oncogenic line D1 or the high oncogenic line D2. Samples of nodule outgrowths transplanted from ovariectomized mice responded to a chemical carcinogen in a similar manner, as did nodule outgrowths transplanted from control experiments. Thus, the altered hormonal environment in ovariectomized mice apparently did not select from subpopulations of nodule cells with altered tumorigenic potentials. The ovarian independence of the nodule lines and tumors derived from them was correlated with a very low level of cytoplasmic estrogen receptor. These results, along with other data, illustrate the nature of the independence on ovarian hormonal control for tumorigenesis in these mammary tumor virus-free nodule lines. (Author Abstract)

74. ESTROGEN-BINDING PROTEINS IN MOUSE MAMMARY TUMORS INDUCED BY DIETHYLSTILBESTROL FEEDING.

Lewko WM, Norvell MJ, Shellenberger TE
Dept. Biochemistry, Univ. Rochester Medical Center, Rochester, NY
Proc Am Assoc Cancer Res; 17:197 1976

Mammary tumors induced by feeding 1000 ppb diethylstilbestrol (DES) to female C3H mice were examined for estrogen binding proteins(ER) using ^{3}H-estradiol-17 beta. Characteristics of these proteins were compared with those of normal mammary gland and uterus using cell-free preparations and either a dextran-coated charcoal procedure of sucrose gradient centrifugation. DES -induced tumors contained ER sedimenting at approximately 7-8 Svedbergs with occasional specimens also exhibiting approximately 4 S species using low ionic strength gradients. Specific binding was insured by incubation of a separate reaction with 500-fold excess of unlabeled DES in the presence of labeled ligand. Specific estrogen binding capacity was estimated from Scatchard analyses of titration data. Specific binding sites were saturated at 2-4 nM ^{3}H-estrodiol and exhibited dissociation constants of 10**-9-10**-10M. Specific binding capacities of mammary tumors ranged from 5-80 fmol/mg cytosol protein while those of normal mammary glands from control and DES-treated females ranged from 20-195 fmol/mg protein. Binding in uteri was up to 10 fold higher. These data demonstrate that mammary tumors induced by DES possess estrogen binding proteins suggesting their estrogen dependence. (Author Abstract)

75. SPECIFICITY OF STEROID BINDING BY THE OESTROGEN RECEPTOR OF RAT MAMMARY TUMOURS INDUCED BY 7,12-DIMETHYLBENZ(A)ANTHRACENE (MEETING ABSTRACT).

Powell-Jones W, Davies P, Wilson DW, Griffiths K
Welsh Natl. Sch. Medicine, Heath Park, Cardiff, CF4 4XX U.K.
J Endocrinol; 68(3):30P 1976

The ability of various natural and synthetic steroids to compete with 2,4,6,7**-3 Hestradiol for protein receptors in 7,12-dimethylbenz(a)anthracene-induced mammary tumors of Sprague-Dawley rats was studied. Specific binding of ^{3} Hestradiol (0.01-10 nM/1) to proteins was studied by sucrose density gradient centrifugation or by charcoal adsorption techniques. At concentrations up to 10,000 times that of ^{3} Hestradiol, the synthetic substances most effective at displacing ^{3} Hestradiol from receptor proteins, when added directly to cytosols, were diethylstilbestrol (DES) and the trans isomer of 1p-(b-dimethylaminoethoxy)phenyl-1,2-diphenylbut-1-ene) (ICI 46,474). After incubation of tissue [illegible] with ^{3} Hestradiol, a ^{3} H-labeled steroid-receptor complex was extracted from purified nuclei. This could be prevented by inclusion of DES and dibutyldihydrostilbestrol. ^{3} HEstradiol binding by receptor proteins was significantly reduced by androst-5-ene-3b,17b-diol, and several other androgenic steroids had varying inhibitory effects. The estrogen receptor apparently has a range of affinities for a number of steroids, including potential androgens, possibly involved in control of estrogen-mediated transcriptional processes in these mammary tumors.

76. ON THE MECHANISM OF HORMONE ACTION IN 7,12 DIMETHYLBENZ(A) ANTHRACENE-INDUCED MAMMARY TUMOR. I. PROLACTIN AND PROGESTERONE EFFECTS ON ESTROGEN RECEPTOR IN VITRO.

Sasaki GH, Leung BS
Univ. Oregon Med. Sch., Portland
Cancer; 35(3):645-651 1975

The presence of estrogen receptors (ER) and the influence of various hormones on the binding capacity of these receptors was studied in 7,12 dimethylbenz(a)anthracene (DMBA)-induced mammary tumors from female Sprague-Dawley rats. Dextran-charcoal assay, Sephadex chromatography, sucrose gradient sedimentation, and organ culture techniques demonstrated the cytoplasmic binding of 3H-estradiol in cytosol preparations from excised tumors. Binding sites were present at 10-13 to 10-15 moles/mg protein, and the dissociation constant was in the range of 10-9 to 10-10 M. Uterine tissue had the highest concentration of ER and nontarget tissue (diaphragm) had the lowest. The presence of prolactin (5 microg/ml) and insulin (5 microg/ml) in the incubation medium significantly increased the estrogen binding capacity. This stimulation was tissue-specific (present in uterine and tumor, absent in diaphragm), and was inhibited by progesterone (1 microg/ml). Insulin and prolactin acted synergistically. It is suggested that the interaction of prolactin, estrogen, and progesterone may be a common phenomenon for all estrogen-responsive tissues.

77. EFFECTS OF OESTRADIOL-17B AND ICI 46,474 ON TOTAL AND ACCESSIBLE CYTOPLASMIC OESTRADIOL-17B RECEPTORS IN DIMETHYLBENZANTHRACENE-INDUCED RAT MAMMARY TUMOURS (MEETING ABSTRACT).

Nicholson RI, Golder MP, Davies P, Griffiths K
Tenovus Inst. Cancer Res., Welsh Natl. Sch. Medicine, Heath, Cardiff, CF 4 4XX, Wales
J Endocrinol; 69(3):50P-51P 1976

The association of the estradiol-17b molecule to its specific cytoplasmic binding protein and the subsequent translocation of the complex to the nucleus are thought to be fundamental in the action of estradiol-17b. Compounds, which interfere with these events, prevent estrogens from exerting their full influence on target tissues. This paper describes the influence of the anti-estrogen, ICI 46,474 (tamoxifen), on the binding of estradiol-17b to total and accessible cytoplasmic binding sites in 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors - an experimental model system for human breast cancer. It was hoped that such studies would provide information on the mechanisms by which anti-estrogens influence tumor growth. The rate of estradiol-17b dissociation from the cytoplasmic estrogen binding protein of DMBA-induced rat mammary tumors and the receptor thermostability were determined at 4, 10, 15, 25 and 37 C. During a 24-hr incubation period, filled sites were labile at either 25 or 37 C. The optimum temperature of dissociation of specifically bound estradiol-17b or ICI 46,474 was shown to be 15 C (total sites). At this temperature the minimum rate of degradation of specific estradiol-17b binding sites was also observed. Sites accessible to estradiol-17b or ICI 46,474 were determined at 4 C. After administration of either estradiol-17b (5 ug) or ICI 46,474 (100 ug) in vivo accessible and total estradiol-17b receptor sites initially decreased in concentration. The effect was followed by a period of increased receptor concentrations lasting approx 5 hr for ICI 46,474 and 48 hr for estradiol-17b. No detectable estradiol-17b binding capacity (total or accessible) was observed 24 or 48 hr after ICI 46,474 administration. The results suggest that the effect of the anti-

estrogen, ICI 46,474, in vivo is similar to that shown by estradiol-17b in that it promotes an initial synthesis of cytoplasmic estradiol-17b receptor protein. It is however, unable to prolong the stimulatory response.

78. ROLE OF ESTROGEN RECEPTOR AND CYCLIC AMP BINDING PROTEINS IN THE GROWTH CONTROL OF HORMONE-DEPENDENT MAMMARY CARCINOMA (MEETING ABSTRACT).

Bodwin J, Clair T, Cho-Chung YS
NIH, Bethesda, Md. 20014.
Proc Am Assoc Cancer Res; 18:117 1977

Growth of 7,12-dimethylbenz(alpha)anthracene (DMBA)-induced mammary carcinoma is arrested by either ovariectomy or N^6,$O^{2'}$-dibutyryl cyclic adenosine 3',5'-monophosphate (DBcAMP) treatment (10 mg/day/200 g rat, S.C.). When this occurs, a change in the specific binding of estrogen and cyclic AMP to the proteins in the cytosol and nuclei of tumors is observed. Growing tumors possess a high estrogen-binding and low cyclic AMP-binding in their cytosol and nuclei; whereas in the cytosol and nuclei from regressing tumors, cAMP-binding was increased 3 to 5-fold and estrogen-binding was decreased by 50-70%. These changes in estrogen- and cAMP-binding activities began within one day after ovariectomy or DBcAMP treatment and the changes were reversed when resumption of tumor growth was induced by either the injection of estradiol-17beta or cessation of DBcAMP treatment. When DMBA tumors failed to regress after ovariectomy, the change in estrogen- and cAMP-binding activities did not occur. Significant increases in the cAMP level as well as in adenylate cyclase and cAMP-phosphodiesterase activities were also found in regressing tumors with increased cAMP-binding activity. The data suggest an interrelationship between the steroid and cAMP in the growth control of a hormone-dependent mammary tumor. (Author Abstract)

79. COMPETITION BETWEEN STEROIDS FOR HORMONAL RECEPTOR.

Lemon HM, Miller M, Foley JF
U. Nebraska Coll. Med., Omaha
Natl Cancer Inst Monogr; 34:77-83 1971

In intact female rats, implantation of pellets containing estradiol (E2) or estriol (E3) within 24-48 hr before or after dimethylbenzanthracene (DMBA) admin reduced the mammary tumor incidence from 62% (controls) to 22% and 12%, resp. Dehydroepiandrosterone (DHEA) did not affect DMBA carcinogenesis. In oophorectomized (oox) rats, DMBA alone or + estrone (E) or E2 induced no tumors. DMBA + E3, DMBA + E3 + E and DMBA + E3 + E2 induced mammary tumors in 6%, 12% and 10%, resp. The hormones alone induced no mammary tumors and did not affect the growth of established DMBA-induced tumors. Binding of 3H-labeled E2 by some tumors from oox rats was inhibited 20-40% by E3 and unlabeled E2. Rapidly-regressing tumors showed a greater than 10% inhibition of 3H-E2 binding by unlabeled E2, but oox-resistant tumors usually contained no estrogenic receptor proteins. E3 was admin. (2.5-10 mg/d) to 8 women with advanced breast cancer. The presence of E3-binding proteins in some human breast tumors was indicated by the signs of tumor exacerbation observed in 2/8 patients. Two other patients, who showed no signs of hormone dependence by any other clinical test, tolerated 2.5-5.0 mg/d of E3 for up to 2 mo without showing definite evidence of either remission or exacerbation. Responses to E3 in the other 4 patients are not described. It is concluded that E3 has an anticarcinogenic effect, but that there is no convincing experimental or clinical evidence that E can inhibit the growth of established, invasive mammary tumors.

80. MAMMARY CARCINOMA: A SPECIFIC BIOCHEMICAL DEFECT IN AUTONOMOUS TUMORS.

McGuire WL, Jennings A, Chamness GC
U. Texas Med. Sch., San Antonio
Science; 175(4019):335-336 1972

Rat mammary carcinoma (R3230AC) has a non-regression characteristic following ovariectomy; in vitro studies are completed using chromatin prepared from A3230AC tumor nuclei with and without estradiol binding protein (EBP) to verify this fact. For testing purposes, the ratios of histone protein to DNA and that of nonhistone protein to DNA were 0.09 to 1.72 and 1.11 to 1.42 respectively. Cytosol fractions from rat muscle, brain, uterus and R3230AC tumor were prepared from the 105,000g supernatant of tissue homogenates and incubated with 17beta6,7-3H estradiol for 60 minutes at 4 C. The complex of 3HE2 and cytoplasm from R3230AC tumor, muscle and brain failed to bind to R3230AC chromatin. This lack of binding ability is attributed to the lack of EBP. To substantiate this result additional testing of rat uterine cytosol, which has abundant EBP, was completed. Analysis of test results in both phases of the experiment showed that EBP is lost during neoplastic transformation, and it is proposed that this loss inhibits normal estrogen action in tissues. Identification of the specific component in EBP which participates in this interaction has not been made.

81. SPECIFIC ESTROGEN-RECEPTORS IN THE NEOPLASTIC AND LACTATING MAMMARY GLAND OF THE RAT.

Wittcliff JL, Gardner DG, Battema WL, Gilbert PJ
U. Rochester Sch. Med. Dentistry, N.Y.
Biochem Biophys Res Commun; 48(1):119-125 1972

Specific 3H estradiol 17beta receptors were studied by a sucrose gradient assay of cytosol fractions from homogenates of normal lactating mammary gland and the estrogen-responsive R3230AC rat mammary adenocarcinoma. Specific estrogen-binding substances, sedimenting as 8S particles, were detected in both normal lactating breast and mammary adenocarcinoma. Preincubation of the cytosol fractions with the antiuterotropic substance CN-55, 945-27 prevented binding of 3H-estradiol. The 8S receptors of both tissues were saturated at estradiol concentrations of 1-2 nM. The estrogen receptors in the hyperplastic and neoplastic tissues exhibited similar dissociation constants of approximately 1 x 10**-9 M. Lactating mammary gland contained about three times as many receptors per mg protein as the mammary adenocarcinoma. Ovariectomy of the host had no apparent effect on the number of binding sites per mg protein in the R3230AC tumor. Progesterone, hydrocortisone, testosterone and dihydrotestosterone did not bind to the estrogen receptors of either lactating mammary gland or R3230AC tumor.

82. ESTRADIOL RECEPTORS IN MOUSE MAMMARY TUMORS: ABSENCE OF THE TRANSFER OF BOUND ESTRADIOL FROM THE CYTOPLASM TO THE NUCLEUS.

Shyamala G
Dept. Zool., U. California, Berkeley
Biochem Biophys Res Commun; 46(4):1623-1630 1972

Spontaneous estradiol-independent mammary tumors were taken from GRS/A mice, incubated with 6,7-3H-estradiol-17beta (3H-E2), homogenized, and tested by sucrose density centrifugation for cytoplasmic and nuclear binding of estradiol in tumor cells. Two discrete estradiol binding components were seen on sucrose density gradients; one had a sedimentation coefficient of 4-5S, the other of 8S. Total binding capacity for the 8S estradiol receptor was 0.02 pmoles/100 microg DNA. Treatment of the tumor cytoplasm with proteolytic enzymes abolished 3H-E2 binding, suggesting

that the binding component was a protein. In these and other respects, the binding characteristics of the 8S component were similar to those of the 8S estradiol receptor complex of the uterus. Tumor cells, however, showed no significant intranuclear binding of 3H-E2.

83. ESTROGEN RECEPTOR CONTENT AND HORMONE-RESPONSIVE GROWTH OF MOUSE MAMMARY TUMORS.

Sluyser M, Van Nie R
Netherlands Cancer Inst., Amsterdam
Cancer Res; 34(12):3253-3257 1974

Mammary tumors induced in ovariectomized GR/A mice by treatment with estrone (po) or progesterone (sc) were transplanted serially into castrated GR/A mice which were left untreated with estrone or progesterone. Estrogen receptor levels in the transplanted tumor were determined by measuring estradiol-3H binding in the cytosol fraction of tumor homogenates. Hormone treated mice showed the most rapid tumor growth following transplantation, (tumors reaching a weight of 2.5 g in 19 days). Estrogen binding was high in treated mice, and low in untreated mice. Transplant generations five through eight grew quickly reaching 2 g in 15 days in the presence and absence of hormone, and estrogen binding in both groups was low. It is suggested that in this system, only tumor cells that are lacking in estrogen receptors can multiply in the absence of progesterone and estrone. Data indicates that hormone-responsive mammary tumors in GR mice are mixed populations of hormone-dependent and hormone-independent cells.

84. BIOCHEMICAL CHANGES IN GR MOUSE MAMMARY TISSUE DURING HORMONAL TUMOR INDUCTION.

Daehnfeldt JL, Schulein M, Briand P
Fibiger Laboratory, Ndr. Frihavnsgade 70, 2100 Kobenhavn, Denmark
Eur J Cancer; 11(7):509-515 1975

Changes in glucose-6-phosphate dehydrogenase (G-6-PDH) and lactose synthetase (LS) activity, and in hormone receptor capacity, occurring during hormonal induction of GR mouse mammary tumors were studied; these parameters were compared in tumorous and nontumorous tissue from the same animals. Treatment of spayed 10-12 wk-old virgin GR mice with estrone (0.5 microg/ml in drinking water) and progesterone (5-10 mg sc pellets weekly) led to mammary tumor development after about 12 wk. Pronounced tissue changes, however, were observed during the first three weeks after the start of hormonal treatment. The total weight of the glands more than doubled during this period, while the protein DNA ratio decreased. G-6-PDH activity rose by 25% during the first two weeks of hormonal treatment calculated on a protein basis, but fell again to the initial value during the remainder of the induction period. LS A-protein activity and B-protein content rose to a steadily high level after two weeks of treatment. The non-blocked cytoplasmic estradiol binding capacity decreased after hormonal treatment of spayed virgins. In the induced tumors, the RNA /DNA ratio was unchanged, and the protein/DNA ratio decreased by 25% compared to nontumorous mammary tissue from the same mice. The activity of G-6-PDH increased by 50%, as calculated on a protein basis. The increased LS A-protein activity remained unchanged, while LS B-protein disappeared. There was ten times as much non-blocked cytoplasmic estradiol receptor protein as in the nontumorous tissue from the same animals. These experiments show that high estradiol receptor content and undetectable levels of LS B-protein are characteristic changes in the hormone-induced GR mouse mammary tumors.

85. HORMONE DEPENDENCE AND ESTRADIOL RECEPTORS IN THE D SERIES OF MAMMARY NODULE OUTGROWTH LINES AND TUMORS.

Medina D, Iramain CA, Clark JH
Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77025
Cancer Res; 35(9):2355-2360 1975

Preneoplastic mammary nodule outgrowth lines were examined for their ability to grow and produce tumors in ovariectomized BALB/c mice. In addition, these nodule outgrowth lines and tumors derived from them were investigated for cytoplasmic estrogen receptor proteins by qualitative and quantitative techniques. Early ovariectomy, performed within 4 weeks after transplantation, slightly delayed but did not permanently block the ability of three different nodule lines to completely fill the fat pad with nodule tissue. Ovariectomy performed later than 4 weeks after transplantation or adrenalectomy performed early or late had no effect on nodule growth. Neither early nor late ovariectomy or adrenalectomy had an effect on the maintenance of the nodule alveolar phenotype. Ovariectomy performed 3 weeks after transplantation had little effect on the tumor potential of the low oncogenic line D1 or the high oncogenic line D2. Samples of nodule outgrowths transplanted from ovariectomized mice responded to a chemical carcinogen in a similar manner, as did nodule outgrowths transplanted from control experiments. Thus, the altered hormonal environment in ovariectomized mice apparently did not select for subpopulations of nodule cells with altered tumorigenic potentials. The ovarian independence of the nodule lines and tumors derived from them was correlated with a very low level of cytoplasmic estrogen receptor. These results, along with other data, illustrate the nature of the independence on ovarian hormonal control for tumorigenesis in these mammary tumor virus-free nodule lines.

86. REGULATION OF ESTROGEN RECEPTORS IN OVARIAN-DEPENDENT RAT MAMMARY TUMORS. I. EFFECTS OF CASTRATION AND PROLACTIN.

Vignon F, Rochefort H
Unite d'Endocrinologie Cellulaire et Moleculaire, I.N.S.E.R.M. U 148, Univ. de Montpellier, Avenue des Moulins, 34000 Montpellier, France
Endocrinology; 98(3):722-729 1976

Ovarian dependent rat mammary tumors, induced by 9,10 dimethyl-1,2-benzanthracene in female Sprague-Dawley rats, were assayed for their estrogen receptor content by dextran-coated charcoal adsorption and sucrose gradient ultracentrifugation. The estradiol receptors bound estrogens with a high affinity, limited capacity and high specificity, and sedimented at 8 S in a sucrose gradient. The cytosol receptors were transferred to the nucleus after binding to estrogens either in vivo or in vitro. The tumor area regressed by 70% during the first 10 days after spaying while the concentration of estradiol cytosol receptors decreased from 225 to 16 fmoles/mg of protein. Three to five days after in vivo administration of estradiol (2 ug daily) or prolactin (1 mg daily) the concentration of estrogen receptors was increased in spayed rats. In biopsy experiments, prolactin, but not estradiol, increased the estrogen receptor concentration when endogenous prolactin release was blocked by CB 154. Prolactin did not modify the intracellular distribution of the estrogen receptor or its binding affinity for estrogen. The uterine estrogen receptor sites were not modified by prolactin under the same conditions. It is suggested that in mammary tumors prolactin sensitizes the action of estrogens at the target level by increasing the concentration of their available receptor sites.

87. MACROMOLECULAR BINDING OF ESTRADIOL BY NUCLEI IN A CELL-FREE SYSTEM.

Haselbacher GK, Eisenfeld AJ

Department of Pharmacology, Yale School of Medicine, New Haven, CT 06510, U.S.A.

Biochem Pharmacol; 25(23):2571-2581 1976

Rat uterine cytosol estradiol-binding protein was labeled with radioactive estradiol and then incubated with highly purified nuclei from an estrogen-binding murine tumor. The nuclei were then extracted with 2 M NaCl-5 M urea, and macromolecular bound estradiol was separated by gel filtration. Nuclear preparations incubated with estradiol-labeled uterine cytosol showed five to ten times more macromolecular binding than corresponding nuclear preparations incubated with buffer, albumin, heart cytosol or uterine cytosol deficient in biologically active binding protein. The process by which nuclei bind estradiol is temperature dependent. Radioactive estradiol attached to protein can be extracted from the nuclei with solutions of 2 M NaCl-5 M urea. Binding of estradiol to isolated nuclei could be distinguished from the binding of the hormone to isolated microsomes or mitochondria in that only the nuclear binding was markedly reduced by prewarming the particulate fraction. Furthermore, nuclear binding was decreased by incubation with several metabolic inhibitors. Most of the nuclear binding appears to occur with the chromatin fraction from which labeled protein-bound estradiol can be extracted. When the cytosol estradiol-binding protein was partially purified by gel filtration there was less nuclear binding of estradiol than resulted from using cytosol. The deficiency of the gel filtration preparation was restored by the addition of unfractionated cytosol. Nuclear binding of estradiol seems to depend on some cofactor present in the cytosol. Nuclei from an estrogen-dependent tumor and the uterus had comparable amounts of nuclear binding that was associated with the 5S fraction as determined by ultracentrifugation on sucrose gradients. Nuclei from liver bound 1/3 as much estradiol, which was associated with a 3S macromolecular fraction. The nuclear-binding macromolecule in the liver is different from that in the uterus and the dependent tumor. Prior incubation of nuclei with non-radioactive estradiol, diethylstilbestrol or estriol in uterine cytosol reduced the uptake of the nuclei when subsequently incubated in (^{3}H)estradiol containing cytosol. Incubation in cytosol containing MER-25, an anti-estrogen, resulted in no subsequent reduction of (^{3}H)estradiol uptake. (Author Abstract)

88. SPECIFIC BINDING OF ^{3}H-OESTRADIOL BY CYTOPLASMIC COMPONENTS OF FEMALE RAT LIVER.

Powell-Jones W, Davies P, Griffiths K

Welsh Natl. Sch. Medicine, Heath Park, Cardiff, CF4 4XX, Wales

J Endocrinol; 69(1):167-168 1976

Mammary tumors were induced in virgin female Sprague-Dawley rats, and specific estradiol binding components in the cytoplasmic extracts were analyzed. Saturation analysis was performed by incubating aliquots of cytosol with varying concentrations (45-1,000 pmol/l) of 2,4,6,7**-3 H-estradiol with and without a hundredfold excess of diethylstilbestrol. Sedimentation analysis of the preparations showed two peaks of radioactivity corresponding to labeled steroid-protein complexes of around 4S and 8S. The 8S peak could be displaced by a hundredfold excess of unlabeled estradiol or diethylstilbestrol with a concomitant increase in the 4S peak. No displacement occurred with high concentrations of progesterone, 5a-dihydrotestosterone, or dexamethasone. Liver cytosols from normal and tumor-bearing rats showed similar patterns. Saturation analysis of the binding of estradiol to low capacity components indicated a dissociation constant of the steroid-protein complex in the range of 900-2400 pmol/l and a receptor concentration of 50-100 fmol/gl of protein.

C. The Estrogen Receptor in Tissues Other Than Breast Cancer

89. IN VITRO STUDIES ON ENDOMETRIUM, ENDOMETRIAL AND OVARIAN CARCINOMA. (PP. 179-193)

Crocker SG, Milton PJ, Taylor RW, King RJ

Gynaecological Malignancy, Clinical and Experimental Studies. Edited by MG Brush, RW Taylor Williams and Wilkins, Baltimore, 1975.

The content of nuclear and cytoplasmic progesterone and estradiol receptors has been investigated in normal human endometrial tissue throughout the menstrual cycle and in endometrial cystic hyperplasia and endometrial carcinoma tissues. Estradiol receptors were observed in the cytosol and nucleus of normal tissue; they were present in large amounts during the follicular phase of the ovarian cycle (with a maximum near ovulation), followed by a decline in the luteal phase. Estradiol binding levels were approximately 0.5 unit for days 1-10 of the cycle, 1.8 units for days 11-14, 1.2 units for days 15-21, and 0.8 unit for days 22-28. Cystic hyperplasia tissue gave levels in the lower range of normal values (mean 0.7 unit for 12 cases), irrespective of the duration of amenorrhea. The values for carcinoma were also in the normal range (mean 1.3 units for 11 cases), irrespective of the state of tumor differentiation. All cases of primary and secondary ovarian carcinomas, two cases of cervical carcinoma, and one ovarian fibroma showed no significant binding. One adenoacanthoma of the endometrium showed binding in the low normal range. Assays of nuclear receptor levels demonstrated the same trends as those for cytoplasmic receptors, suggesting that the nuclear uptake of estradiol depends on the availability of cytoplasmic receptors. A specific 5S-8S receptor complex for progesterone was found in large amounts (4-13 units) in normal endometrium during the follicular phase, with a fall in the luteal phase (to 0.3-7 units). In cases of cystic hyperplasia the mean receptor content was comparable with that of normal tissues in the follicular phase, reflecting either prolonged but mild estrogen stimulation or the absence of endogenous progesterone capable of blocking available sites. Endometrial carcinoma tissues from both pre- or postmenopausal patients contained progesterone receptors, with the exception of one anaplastic tumor. The receptor content seemed to be related to the state of tumor differentiation and may reflect tumor responsiveness to progestogen therapy.

90. ESTRADIOL BINDING BY HUMAN ENDOMETRIAL TISSUE.

Muechler EK, Flickinger GL, Mangan CE, Mikhail G

Sch. Medicine, Univ. Pennsylvania, Philadelphia, PA

Gynecol Oncol; 3(3):244-250 1975

A quantitative dextran-coated charcoal assay was used to measure estrogen binding by human endometrium in various hormonal states. Following incubation of cytosols (supernate from 105,000 x g for 60 min) with H3-estradiol, the amount of hormone bound is expressed as 10-15 mole DNA and 10**-15 mole/mg protein. Proliferative endometrium gave the highest values (1.95 and 244, respectively), while lowest levels (0.68 and 49.2) were found for atrophic endometrium. Endometrium from patients on oral contraceptives had low values (0.71 and 60.4), as was the case with secretory endometrium (0.77 and 90.9). The most consistent results were found in areas of hyperplastic endometrium (1.51 and 139). Estrogen binding by cytosols prepared from 17 cases of endometrial adenocarcinoma and 1 case of endocervical

adenosquamous carcinoma was variable: six showed no detectable binding, seven had low values, and in five the binding was elevated. A wide range of receptor levels was observed in tissues from different areas of the same uterus. There was no particular correlation between tumor differentiation and receptor level. Controlled studies are suggested to determine whether estrogen binding level is related to response to hormone treatment. (35 refs)

91. OESTROGENS AS A CAUSE OF ENDOMETRIAL CARCINOMA.

Anonymous
No affiliation given
Br Med J; 1(6013):791-792 1976

Postmenopausal women under hormone treatment excrete measurable amounts of estrogen, probably by the peripheral conversion of androstenedione to estrone. It has been postulated that the exclusive production of estrone rather than the normal ovarian hormone, estradiol-17b, might lead to an increased risk of endometrial cancer. In a recent study of the therapeutic history of 94 patients with endometrial carcinoma, it was found that 57% of the cancer patients had been treated with estrogens (estrone sulfate) compared with 15% of matched controls. Other studies supported this finding of increased risk of endometrial cancer in estrogen-treated women. Alternative formulations should be found for hormone replacement therapy. Suggested alternatives are estriol, which has a weak effect on the endometrium and decreases the biological effect of endogenous estrone, and estradiol-17b, which would be taken up preferentially (over estrone) by the binding protein in the endometrial cell cytoplasm.

92. ESTROGEN RECEPTOR IN HUMAN MYOMA TISSUE.

Puukka MJ, Kontula KK, Kauppila AJ, Janne OA, Vihko RK
Dept. Clinical Chemistry, Univ. Oulu, SF-90220 Oulu 22, Finland
Mol Cell Endocrinol; 6(1):35-46 1976

The occurrence and characteristics of an estrogen receptor in the cytosol of myoma samples from human uteri (patients aged 30-72 yr) were investigated, employing dextran-coated charcoal and density gradient centrifugation techniques. Receptor binding site concentrations in 24 myoma specimens ranged from 23 to 515 femtomoles/ml cytosol protein (mean: 98 +-108). In one myoma sample no receptor was found. The apparent equilibrium dissociation constant was 1.3 x 10**-10 moles/liter for estradiol-17b. On sucrose density gradient centrifugation, H3-estradiol was bound by macromolecules with sedimentation rates of 4 and 8 S. The latter component was specific for estrogens, whereas the former contained specific and nonspecific binding sites. Ligand specificity studies were carried out utilizing 30 different steroidal compounds. A good correlation was found between the in-vitro binding affinity and the in-vivo estrogenic potency of the compounds tested. The cytosol estrogen receptor from myoma had a ligand specificity closely resembling that of the corresponding receptor both in normal human myometrium and endometrium, and in human breast carcinoma. The myoma estrogen receptor level was compared to that in normal myometrium and endometrium in 13 uterine specimens. The receptor concentrations in cytosol fractions from myoma and myometrium correlated significantly, whereas no correlation existed between the receptor levels in endometrial and myoma cytosols. Furthermore, the estrogen receptor content in myoma samples did not correlate to estradiol-17b levels in the myoma cytosol or serum of the same patient. It appears that when either a benign or malignant neoplastic transformation occurs in a human estrogen-dependent tissue, there is no significant alteration in the cytosol estrogen receptor, at least not in that portion which is adjacent to the steroid-binding site. (33 refs)

93. STEROID HORMONE REGULATION OF GENE EXPRESSION.

O'Malley BW
Baylor College of Med., Houston, Texas
Cancer Biology; I. Induction, Regulation, Immunology and Therapy. Based on a Series of Lectures Presented at the Given Institute of Pathobiology in Aspen, Colorado, July 1974. Edited by R.R. Pascal, F. Silva and D.W. King. (New York: Stratton Intercontinental Medical Book Corp.):34-37 1976

The effects of steroid hormones on the regulation of gene expression with the chick oviduct as a model, are reviewed. A steroid hormone receptor in chick oviduct cells has been purified to homogeneity and characterized as having two protein molecules, each of which possesses a steroid binding site and one of which also possesses a DNA binding site. Chick oviduct shows specific responses to estrogen and progesterone. In the newly hatched chick, oviduct growth and development is dependent upon estrogen, as is the synthesis of ovalbumin. Progesterone produces no growth changes, but is required for the synthesis of avidin. The administration of diethylstilbestrol (DES) to chicks yields both increased intracellular ovalbumin and increased mRNA activity. The increased mRNA activity correlates with the rate of ovalbumin synthesis. Progesterone produces a concurrent increase in mRNA activity and rate of synthesis of avidin. Thus, steroid hormones influence mRNA in their action of cells. Ovalbumin mRNA can be purified 97.5% compared to a whole cell extract, and codes specifically for ovalbumin synthesis. It appears that one gene is responsible for the production of relatively large amounts of specific ovalbumin mRNA. Template activity assays before and after hormone stimulation indicate that "chromosomal proteins maintain binding sites in unstable forms." With DES stimulation of the chromatin, the ability of the template to bind and stack RNA polymerase increases rapidly. The main effect of steroid hormone-receptor complexes appears to be stimulation of transcription at the level of initiation. These findings suggest that some hormones may disrupt normal DNA in such a way as to enhance development of neoplasia.

94. THE EFFECT OF DIETHYLSTILBESTROL ON THE PRENATAL DEVELOPMENT OF CHICK MULLERIAN DUCT (MEETING ABSTRACT).

Teng CS, Teng CT
Dept. Cell Biology, Baylor Coll. Medicine, Houston, TX 77025
J Cell Biol; 70(2/Part2):145a 1976

Sex steroid hormones are involved in the differentiation and growth of Mullerian duct (Md) cell. The interaction of estradiol (E2) with its receptor molecule in the cytoplasm and the E2-receptor complex translocation into nucleus of the embryonic Md cell have already been examined. Yet, the mechanisms of the estrogenic hormone effect on the developing Md cell is still unknown. During the normal development of the embryonic chick Mds, the left duct develops into the functional oviduct and the right one regresses after 12th day of incubation and becomes a rudiment at 15th day of incubation. When non-steroid estrogen, diethylstilbestrol (DES) was administered into the female embryo at 5th day of incubation (approx three days before the initiation of sex tract differentiation), the regression (or death) of the right Md cell was totally prevented and the growth of both Mds was extensively stimulated. The wet weight of Mds from DES-treated embryo was increased about 200% and 400% above control in the left and right Md of the 15-day embryo. Total RNA content in the left and right Md was

increased approx. 190% and 420%, and the total protein content increased about 178% and 456% in the respective left and right Md. Histological observation indicated the precocious differentiation of the epithelial cells in the left and right ducts. As compared to the control Md cell, the cytoplasmic E2 receptor concentration in the DES-treated Mds cell decreased to 16-18% of the original value. No significant change of dissociation value or nuclear E2 binding sites was observed. The above results indicated that during the early stage of sex organ development, the cells in the target organ are fully responsive to estrogenic hormone, and the pattern of cell differentiation and development is regulated by the hormone at the transcriptional and translational levels.

95. ESTRADIOL MUSTARD (NSC-112259) AND PHENESTER (NSC-116785): POSSIBLE MEDIATION OF ACTION BY ESTROGEN BINDING PROTEIN.

Everson RB, Turnell RW, Wittliff JL, Hall TC
Div. Cancer Cause Prev., Natl. Cancer Inst., Bethesda, Md.
Cancer Chemother Rep; 58(3):353-357 1974

Uteri from Fischer 344 rats were cleaned, homogenized in Tris-HCl (10mM), Na(2)EDTA (1.0mM), sucrose (250 mM), buffer and a supernatant was prepared at 50,000 or 105,000 x g. The cytosol preparation was incubated with either 17beta-estradiol (ED), CN-55,945-27 (CN), estradiol mustard (EM) or phenester (PH) at 0-3 degrees C for 10-30 min, then transferred to a vial, ^{3}H-ED was added and incubated at 0-3 degrees C for 14-20 hr. The amount of bound ^{3}H-ED was determined by a modified charcoal binding technique to remove the unbound ^{3}H-ligand; a gradient centrifugation analysis was also performed in some experiments. ED (2 x 10**-7M) and EM (10**-3M), completely inhibited ^{3}H-ED binding; PH had no effect on ^{3}H-ED binding. CN completely inhibits binding of ^{3}H-ED to 8S receptors as determined by sedimentation profile. EM (10**-5M) resulted in a 35% reduction in ^{3}H-ED binding to 8S receptors; there was an 80% reduction at EM (10**-4M). This displacement of binding by EM observed on sucrose gradients and charcoal assays was not due to free estradiol released after breakdown of the EM as the binding of EM was maximal at 3 hr of incubation and did not change through 13.6 hr of incubation. These results suggest that ED binding proteins in the cytoplasm may mediate the action of EM. (14 refs)

96. EARLY STAGES IN ESTROGEN CONTROL OF CELL PROLIFERATION.

Bresciani F, Puca GA, Nola E, Sica V
Fac. Med. Surg., Univ. Naples, Italy
Cold Spring Harbor Conf Cell Proliferation; 1:67-83 1974

The early stages of 17beta-estradiol interaction with the uterus (1) association of the incoming hormone with a specific receptor in the cytoplasm, (2) transformation and translocation of the estrogen-receptor complex to the nucleus, and (3) interaction of the complex with chromatin are reviewed. Studies on calf uterus resulted in the identification of a receptor-transforming factor (RTF) of cytoplasm that acts on the native estrogen-receptor complex (5.3S) to produce a smaller complex (4.5S) which is found in the nucleus. The RTF has been partially purified. It is a protein which sediments at 6.4S, has a Stokes radius of 46 Angstroms, a molecular weight of 115,000, and an isoelectric point of 4.9. It has a high affinity for the native estrogen-receptor complex and shows a Ca++-dependent proteolytic activity on alpha-casein but not on native or denatured bovine plasma albumin, ovalbumin, or hemoglobin. The pH optimum is 8.7. The RTF-induced size decrease of the native estrogen-receptor complex appears to be a case of limited hydrolysis and may be instrumental in the cytoplasm to nucleus translocation of the protein-bound hormones. To identify nuclear acceptor sites for the estradiol-receptor complex, nuclear components in calf uterus fractions were coupled with CNBr-activated Sepharose. Nuclear binding sites were identified which have an affinity in excess of 109 liters/mole (+4 Cl for estrogen-receptor complex, but not for free receptor, at 0.1 M KCl. These sites are protein in nature, are soluble at 2 M NaCl, pH 7.1, but precipitate when KCl concentration is decreased to 0.1 M. They are not retained by hydroxyapatite at pH 7.0 but are retained by carboxymethyl (CM0-cellulose at pH 4.0 or 6.0). Hydrochloric acid (0.03 N) is required for their elution from CM-cellulose. These results indicate that the estrogen-receptor-complex acceptor sites consist of basic protein. Protamine, histone IIa (calf thymus), and alpha-casein coupled to Sepharose also associated with estrogen-receptor complexes but with affinities on the order of 102 liters/mole at +4 C. Based on these findings, it is hypothesized that estrogen enters the cell and interacts with the 5.3S receptor, which is confined to the cytoplasms through association with some cytoplasmic structure. Activation of RTF by Ca++ results in the production of a mobile 4.5S-estrogen fragment, which enters the nucleus and specification interacts with a basic acceptor protein.

97. NUCLEAR AND CYTOPLASMIC OESTROGEN RECEPTORS IN VAGINAL AND UTERINE TISSUE OF MICE TREATED NEONATALLY WITH STEROIDS AND PROLACTIN.

Shyamala G, Bern HA
Univ. California, Berkeley
J Endocrinol; 63(2):275-284 1974

Nuclear and cytoplasmic estrogen receptors in vaginal and uterine tissue were studied in female BALB/cCrgl mice treated neonatally with sc injections of estradiol-17beta (5-20 microg); testosterone (5-20 microg); unlabelled estradiol-17 beta (0.1 microg); or 5 microg estradiol plus 5 microg prolactin. The degree of steroid sensitivity on target tissues was assessed by measurement of the receptor contents. Cornification and any associated physical changes did not have a significant influence on the estrogen receptors. Vaginal estrogen responsiveness (dependence) of mice treated with low and high doses of steroids was correlated with estrogen receptor content. Neonatal treatment with prolactin in combination with low doses of steroids favored development of ovary-dependent cornification and a concomitant decrease in estrogen receptors in about 50% of the mice studied.

98. INTERACTION OF ESTRADIOL WITH A NUCLEAR PROTEIN.

Puca GA, Nola E, Caso P, Sico V
Surgery, Radiotherapy and Chemotherapy of Cancer
Proceedings XI International Cancer Congress Florence 1974. Bucalossi P, Veronesi U, Cascinelli N, ed.
Amsterdam, Exerpta Medica, 1974 1.90022E-56 + +

This is a part of the continuing investigations on the estrogen receptor/nuclear acceptor interaction in the uterus, applying affinity chromatography to identify and characterize the nuclear component (acceptor). Fresh calf uterus nuclei were purified and fractionated. Material extracted from the nuclei with 2 M NaCl was dialyzed against 25 mM sodium phosphate buffer (pH 7.1) and centrifuged at 48,000 for 20 min. The sediment (containing nuclear DNA associated with basic and some acid nuclear protein) was extracted for 20 min at 4 C with 0.1 N HCl; after centrifugation for 20 min at 48,000, the supernatant was adjusted to pH 6.2. Cytoplasmic estradiol receptor was found to have interacted with a specific nuclear fraction in a way that correlates closely with the nuclear binding of estrogen-receptor complex either in vivo or in intact cells in vitro. The interaction with the nuclear fraction showed a high association constant; it depended strictly on the hormone being bound to the receptor. The nuclear acceptor sites appeared to be primarily protein (with a molecular wt of

70,000 daltons), since they are destroyed by proteases and not by nucleases. Furthermore, both native DNA bound to cellulose and denatured DNA bound to agarose did not absorb estrogen-receptor complex. Sulfhydryl groups of the estradiol receptor, but not nuclear acceptor, appeared to have a role in the acceptor-receptor binding.

99. STUDIES ON OESTROGEN-BINDING PROTEINS (OESTROGEN RECEPTORS). (PP. 149-157)

Bresciani F, Nola E, Sica V, Puca GA

Host Environment Interactions in the Etiology of Cancer in Man. Proceedings of a meeting held at Primosten, Yugoslavia, 27 August-2 September 1972. International Agency for Res. on Cancer, Lyon, France, pp. 149-157 1973.

The results of purification and characterization studies of estrogen binding (EB) proteins from prepuberal calf uterus are reviewed. Only three forms of molecular EB protein were recognized. There is an 8.6S, 240,000 mol wt form present in cytosol; this state changes into a 5.3S, 118,000 mol wt form after the ionic strength of the medium is increased. The next step is the formation of a smaller, low-salt, stable 4.5S, 60,000 mol wt protein. It is suggested that formation of the 4.5S EB protein by the receptor-transforming (RT) factor is the step whereby confinement to the cytoplasm of the larger 5.3S-8.6S system is overcome and thereby nuclear penetration of the estrogen-protein complex is allowed. The main properties of the RT-factor are: sedimentation coefficient 6.4; mol wt, 110,000; isoelectric point, 4.9; it is destroyed by proteases but not by nucleases and by heating at 60 C for 20 min; it is present in the 105,000 g supernatant; it is inactive and stable in the absence of $Ca^{2}+$; $Mn^{2}+$ and $Sr^{2}+$ may, in part, replace calcium; and its activity is unaffected by phenylmethylsulfonyl fluoride. It is clear that the RT factor is in part protein and an entity different from the EB proteins. It does not bind estrogen but shows a high affinity for the 5.3S EB protein. Preliminary evidence suggests that the RT factor is a protease; it is not of the trypsin group.

100. A MOLECULAR AND KINETIC ANALYSIS OF ESTROGEN RECEPTOR TRANSFORMATION.

Notides AC, Nielsen S

Dept. Pharmacology and Toxicology, Univ. Rochester Sch. Medicine, Rochester, NY 14642

J Steroid Biochem; 6(3/4):483-486 1975

A molecular and kinetic analysis of estrogen receptor transformation is presented. Recent studies of the uterine estrogen receptor indicate it is a multisubunit protein. Estradiol and temperature induce a conformational change in the 4S estrogen-binding protein (EBP) or monomer (molecular wt 7-8 x 10^{4}), as indicated by the high energy of activation (19-21 kcal mol**-1) accompanying the formation of the 4S EBP dimer, 5S EBP (molecular wt 13-14 x 10^{4}). The formation of 5S EBP suggests that new intramolecular bonding forces have been established, presumably hydrophobic interactions not available in the inactive 4S EBP monomer. Kinetic analysis shows that the 4S-5S EBP transformation is a second-order reaction, dimerization of 4S EBP with a second (similar or dissimilar) subunit. The 5S EBP produced by in vitro warming of the cytosol**-3 H-estradiol and 5S EBP from isolated nuclei have similar molecular weights, sedimentation coefficients, molecular radii, and formation rates. The results suggest that an estradiol- and temperature-induced conformational change in 4S EBP leads to a macromolecular association process and receptor activation. (20 refs)

101. CHARACTERIZATION OF ESTROGEN-BINDING PROTEINS IN SEX STEROID TARGET CELLS GROWING IN LONG-TERM CULTURE.

Soto AM, Rosner AL, Farookhi R, Sonnenschein C

Tufts Univ. Sch. Medicine, Tufts Cancer Res. Center, Boston, MA

Methods Cell Biol; 113:195-211 1976

Methods used to characterize estrogen receptors (ER) in cells growing in long-term culture were evaluated. Established cell lines carrying or not carrying ER were studied; the former included rat pituitary cell lines GH3 , RAP, and FPG, and rat endometrial cell line U15, while the latter included HeLa and mouse myeloma (MPC11) cells. Determination of the properties and molar concentration of estradiol-17b (E2) receptor sites in a subcellular extract was based on the competition for these specific binding sites, by both labeled and unlabeled E2 . After equilibrium, bound E2 is separated from the remaining free steroid by one of four techniques: adsorption of free E2 by charcoal, adsorption of bound E2 by hydroxylapatite, adsorption of bound E2 by diethylaminoethanol (DEAE) filters, and recovery of all bound E2 by protamine sulfate precipitation. Assays of the diminishing amounts of labeled E2 that can be recovered with ER (as increasing amounts of unlabeled steroid hormone are included with the incubations) are carried out. A comparison of the relative efficiencies of each technique in determining the amount and properties of ER of C5U15 subcellular fractions is presented. In the presence of 0.5 M KCl used to extract nuclear ER (receptor II), a slight decrease in the number of moles of recovered receptor II is noted if ER is retrieved by adsorption to DEAE, and a more significant decrease is seen if the charcoal adsorption method is used. The measurement of ER by its precipitation by protamine sulfate is as desirable a method as that involving adsorption of ER to hydroxylapatite. However, the precipitate is extremely fine and becomes highly gelatinous after 15 min at 4 C, and unless specific precautions are taken, loss of material on the walls of glassware is likely. Because the measurement of ER by its adsorption to hydroxylapatite remains the only technique relatively independent of (1) variations in ionic strength (2) different concentrations of protein in the subcellular fraction (3) time of equilibrium with the adsorbant, and (4) possible loss of material on glassware walls, it is the most definitive method for study of the properties of ER in sex steroid target cells. (40 refs)

102. CHARACTERIZATION OF AN ESTROGEN-SENSITIVE CELL LINE ESTABLISHED FROM NORMAL RAT ENDOMETRIUM.

Sonnenschein C, Weiller S, Farookhi R, Soto AM

Tufts U. Sch. Med., Boston, Mass.

Cancer Res; 34(11):3147-3154 1974

The establishment of an estrogen-sensitive cell line derived from endometrial tissue of a prepuberal Wistar-Furth female rat is described. The cell line U15 was established after the primary culture was grown in medium supplemented with estradiol-17beta at 1 x 10**-9 M. Clonal derivatives of this cell line produced tumors at the site of inoculation with histopathological features of adenocarcinoma of the endometrium. These tumors grew significantly better where estradiol-17beta concentrations were either physiological or slightly above physiological levels. Clonal cell lines in culture and tumors developed in situ, when injected into appropriate hosts, showed high affinity and saturable binding proteins for estradiol-17beta, estriol, and estrone, in that order of affinity. The dissociation constant (Kd) for estradiol-17beta is about 2.2 x 10**-11 M, and the number of binding sites was about 6800 per viable cell in culture (6000 in the cytosol and 800 in the nuclear fraction) under the experimental conditions

described. The Kd for tumor preparations was about 1 order of magnitude higher (Kd = 1.3 x 10**-10 M). Further characterization of these U15 cells is expected to help explain the mechanism of action of estradiol-17beta in cloned populations of target cells in genetic terms.

103. ESTROGENIC PROPERTIES OF 3,9-DIHYDROXYBENZ(A)ANTHRACENE, A POTENTIAL METABOLITE OF BENZ(A)ANTHRACENE.

Schneider SL, Alks V, Morreal CE, Sinha DK, Dao TL
Department of Breast Surgery and Endocrine Research Laboratory, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263

J Natl Cancer Inst; 57(6):1351-1354 1976

The estrogen receptor binding properties of 3,9-dihydroxybenz(a)anthracene (3,9-diOHBA) were determined in uterine cytosol of immature Sprague-Dawley rats by competitive binding experiments with (^{3}H)estradiol and sucrose density centrifugation. 3,9-DiOHBA inhibited estradiol binding to the 8S binding protein at a concentration (1.2x10**-5 M) approximately equal to that of nafoxidine-HCl (3x10**-5 M) required to inhibit estradiol-specific binding, and bioassay for estrogenic activity substantiated this finding. (Author Abstract)

104. ALPHA-FETOPROTEIN IS NOT A COMPONENT OF THE ESTRADIOL RECEPTOR OF THE RAT UTERUS.

Radanyi C, Mercier-Bodard C, Secco-Millet C, Baulieu EE, Richard-Foy H
Lab Hormones, 94270-Bicetre, France

Proc Natl Acad Sci USA; 74(6):2269-2272 1977

In high-salt medium, cytosol from immature rat uteri displays two main high-affinity estradiol-binding peaks after ultracentrifugation in a sucrose gradient. The two components are the estradiol receptor which has a sedimentation coefficient of 5.5 S, and the alpha-fetoprotein which sediments at 4.5 S. The dissociation rate constants (k-1) of plasma alpha-fetoprotein-estradiol complexes measured at 0 C in the absence or presence of 0.4 M KCl were found to be 7 x 10**-5 and 8 x 10**-5 sec**-1, respectively. The half-time of dissociation of these hormone-plasma protein complexes is 100-200 times more rapid than that of the estradiol-receptor complexes. These data led to the use of two "differential dissociation" methods for the measurement of the hormone-binding protein complexes. In a high-salt cytosol, the charcoal technique measured selectively the receptor binding sites; the hydroxylapatite technique measured the sum of the alpha-fetoprotein plus receptor binding sites. Under these conditions, binding specificity studies provided evidence that alpha-fetoprotein is not a subunit of the receptor. This was confirmed by binding specificity studies in high-salt medium of the receptor separated from alpha-fetoprotein by ultracentrifugation. (Author abstract) (29 Refs)

105. ESTROGEN DERIVATIVES FOR THE EXTERNAL LOCALIZATION OF ESTROGEN-DEPENDENT MALIGNANCY.

Komai T, Eckelman WC, Johnsonbaugh RE, Mazaitis A, Kubota H, Reba RC
Div. of Nuclear Medicine, George Washington Univ, Washington, DC 20037.

J Nucl Med; 18(4):360-366 1977

Four radioiodinated estrogen derivatives were studied to determine their affinity for the estrogen-binding protein found in the cytosol of rabbit and rat uteri. In vitro determination of the binding properties by competitive-binding experiments and by sucrose-gradient centrifugation indicates that one of the derivatives, iodohexestrol, binds to the cytosol estrogen-binding protein. This in vitro behavior was related to in vivo distribution. Studies in immature female rats showed high uterine uptake of iodohexestrol at 2 hr (1.69% dose/gm). Iodohexestrol also has a high nonspecific binding in both the blood and the uterine cytosol. Thyroxine can diminish the nonspecific binding in vitro; in vivo the prior injection of thyroxine increased the 2-hr uterus-to-blood ratio from 1.9 to 10.4. The in vitro receptor-assay system was helpful in predicting in vivo distribution. (17 Refs) (Author Abstract)

106. STEROID RECEPTORS AND HORMONE RECEPTIVITY: NEW APPROACHES IN PHARMACOLOGY AND THERAPEUTICS. (PP. 165-176)

Baulieu EE

Breast Cancer, Trends in Research and Treatment A Monograph of the European Organization for Research on Treatment of Cancer. New York, Raven Press, 1976.

A review is presented of steroid receptors, with reference to definitions and studies; physiological changes of receptor content and receptivity; different receptors for the same hormone in different target cells, for different hormones in the same cells, and for the same hormone in the same cells; and new therapeutic approaches. Evidence was gathered that indicated that the specific proteins binding selectivity to the corresponding hormones of a given tissue are receptors, capable of recognition and operational in message execution. Studies have been conducted to determine if their qualitative and quantitative evaluation may be of practical value for a better understanding of hormone receptivity and if their pharmacological manipulation can contribute to better control of cellular functioning in intact organisms. Physiological and hormone-induced changes of receptor concentrations have been demonstrated (increase of progesterone receptor during proestrus in the guinea pig; changes in uterine estrogen receptor during early pregnancy in the rat), and will probably be seen in other tissues and circumstances. It is unknown whether receptors for a hormone are the same in different target tissues, although there is one example showing that a hormone (testosterone) circulating in the blood may play its part in different tissues through different receptors. There are also different receptors for different hormones in the same cells; cloned cells (M11) from a mammary tumor SHI-115 in mice possess two receptors, estrogen and androgen, binding estradiol and testosterone, respectively. Estradiol, however, can bind to both receptors, thus demonstrating that two different receptors can bind the same steroid, depending on the concentration. Diethylstilbestrol (DES), a nonsteroidal estrogen, also binds to the estrogen receptor in a way that is similar to the estradiol itself, while its interaction with the androgen receptor is very weak. Based on these data, it is noted that receptivity to a given hormone may depend on the amount of corresponding receptor in target cells. There are good correlations between hormonal response of breast cancer and the estradiol receptor content, and of lymphoblastic leukemia and corticosteroid receptors, but results of pharmacological manipulations are yet unknown. Induction of steroid receptor by steroid hormones is of great value for explaining priming, but negative influence of steroid on receptor induction is one way to explain certain antagonisms between hormones, other possibilities being the simultaneous presence of different receptors or competition for the same receptor. Other results (mediation of testosterone effects; different effects of DES and estradiol) may also lead to new therapeutic advances. (45 refs)

107. SOME ASPECTS OF THE MECHANISM OF ACTION OF STEROID HORMONES.

Baulieu EE

Lab Hormones, 94270-Bicetre, France

Mol Cell Biochem; 7(3):157-174 1975

Early steps in sex hormone action, reversible systems such as the growth promoting effect and the study of steroid transport to the receptor site are reviewed. The proportion graph methodology, a log plot permitting expansion of the scale of representation of the bound ligand to include several orders of magnitude of total hormone conc is discussed. Various other methods for measurement and calculation of protein and steroid interactions include differential dissociation and kinetics measurements of the association and dissociation rate constants. The steroid-binding plasma proteins are listed and their physiological variations and possible roles are discussed. The mechanism of steroid entry into target cells is then considered, especially the possibility of a protein-mediated step. The metabolism of steroid hormone in target cells and its physiological significance are discussed, especially with reference to androgens in prostate, levator ani muscle and hypothalamus. Specific proteins (receptors) have been detected in the target tissues of steroid hormones, with properties that are compatible with an essential role in steroid action. The properties ascribed to the cytosol receptors of different steroid hormones in various tissues, their binding properties and their quantitative variations are reported. The transformation or activation of receptor after binding of the hormone is related to its transfer to the nucleus. The question posed by the recent observations on non-histone chromatin estrogen binding protein and of the insoluble nuclear receptor are discussed. A series of experiments dealing specifically with the increase of protein and RNA synthesis in tissues in which steroids promote growth estradiol in uterus are reviewed. The concept of a cascade phenomenon is reported, including the early synthesis of key intermediary proteins which would be responsible for the secondary amplification of the response. This is discussed in connection with the present knowledge of receptors and transcription machinery.

108. HORMONAL RECEPTORS.

Baulieu EE

Bicetre Hosp., France

Nouv Rev Fr Hematol; 7(5):589-599 1967

A specificity of estradiol (E) binding was demonstrated by supernatant proteins from cells of immature sow endometrium according to the following parameters: absence of competition at physiologic levels for binding by these proteins between E, diethylstilbestrol and other hormones such as antidiuretic hormone, testosterone, 19-nortestosterone, hydrocortisone or progesterone; quantitative evidence of a limiting point for the binding capacity of these receptors to physiologic E concn and, under similar conditions, pulmonary tissue completely lacked such a binding system. It is suggested that the phenol nucleus is indispensable for this binding system and that the 17BETA-hydroxy group also plays an important role in binding

109. ESTROGEN-INDUCED LEYDIG CELL TUMOR IN THE MOUSE: A MODEL SYSTEM FOR THE STUDY OF CARCINOGENESIS AND HORMONE DEPENDENCY.

Huseby RA

American Cancer Res. Center Hosp., 6401 West Colfax Ave., Lakewood, CO 80214

J Toxicol Environ Health; 1:177-192 1976

The estrogen-induced Leydig cell tumor in the mouse is described. Since pituitary function is necessary for Leydig cell tumor development, a reduced level of hypophyseal function may be a contributing factor to the lack of tumor development. The dependence of induced Leydig cell tumors on pituitary function as they relate to growth and function may represent independent phenomena. Similarly, dependency on estrogen stimulation and upon hypophyseal function are independent of the other. Leydig cells have specific protein receptors in the cytoplasm that bind estrogen and transport it to the nucleus where it is also bound. Surgically confining the testes to the abdominal cavity increases the propensity to develop Leydig cell tumors. It is concluded that the induced Leydig cell represents a very useful model system for the study of both endocrine carcinogenesis and endocrine tumor dependency. (28 refs)

110. ESTROGEN RECEPTOR CHARACTERIZATION OF THE R3327 TRANSPLANTABLE PROSTATIC ADENOCARCINOMA. (MEETING ABSTRACT).

Markland FS, Lee L

Dept of Biochemistry USC Sch of Medicine, Los Angeles, CA 90033

Fed Proc; 36(3):913 1977

The R3327 prostatic adenocarcinoma is a slow growing, hormonally responsive tumor that is carried by subcutaneous transplantation in intact male Copenhagen rats. We report here results of studies on the characteristics of the tumor's steroid hormone receptors. Tumors were removed 7-9 months after transplantation and stored in liquid N2. Cytosol was prepared and incubated with 'H-estradiol-17beta ('H-E2) in the presence or absence of excess radioinert E2. Receptor content was analyzed by sucrose density gradient centrifugation in low salt (SG) which revealed suppressible binding of 15-30 fmoles/mg cytosol protein (fm/mg) in the 8S region. Scatchard analysis indicated a single class of high affinity binding sites with a Kd of the order of 5x10**-10M, and a total binding capacity of 20-35 fm/mg. Receptor interaction with 'H-E2 was highly specific since binding in the 8S region was completely inhibited by 100 fold excess radioinert E2 or diethylstilbestrol. However, similar excess of a synthetic androgen, or progestin, or glucocorticoid did not inhibit binding significantly. By contrast, plasma from male Copenhagen rats contains no suppressible E2 binding as analyzed by SG. The tumor also contains a specific androgen receptor which sediments in the 8S region on SG. The presence of steroid hormone receptors explains the hormonal sensitivity of the tumor. (Author Abstract)

111. RECEPTORS FOR STEROID HORMONES IN HUMAN MELANOMA.

Neifeld JP, Lippman ME, Fisher RI

Surgery Branch, NCI, NIH, Bethesda, MD 20014

Surg Forum; 27:108-110 1976

Human melanoma samples were evaluated for steroid hormone receptors. Assays were performed within 2 wk of sample collection. A dextran-coated charcoal assay was used to determine the estrogen, progesterone, and glucocorticoid-binding activity, and a protamine sulfate assay was used to determine androgen-binding activity. A total of 35 patients had tumor samples assayed for steroid hormone receptor activity: the tumor was from the primary lesion (3 patients), a soft-tissue recurrence (13), a metastatic lymph node (17), a liver metastasis (1), and a pulmonary metastasis (1). Sixteen patients were men, and 19 were women. Estrogen receptor activity was present in 16/35 patients. Scatchard analysis of binding in one case was consistent with a single class of receptor sites with a dissociation constant of approx 4.9 X 10**-9 M and approx 24.7 femtomoles of specific binding per mg of protein. Androgen receptor was observed in 5/30 patients (with 6 equivocal), progesterone receptor in 6/27 patients (with 2 equivocal), and glucocorticoid receptor in 5/26 patients. Estrogen and androgen receptors were found concomitantly in two patients, estrogen and progesterone in

four, estrogen and glucocorticoid in three, and estrogen, androgen, and progesterone in one patient. Five patients with no estrogen receptor had specific binding for another class of steroid hormones. The investigation has demonstrated that specific steroid hormone receptors are present in human melanoma. The use of hormone receptor analyses may permit selection of a subset of patients with disseminated melanoma who might be candidates for a trial of hormonal therapy. (5 Refs)

112. ESTROGEN RECEPTORS IN HAMSTER MELANOMA, HM MEL 1. (MEETING ABSTRACT).

Snyder J, Das Gupta TK
Div of Surgical Oncology, Univ of Illinois Med Sch, Chicago, IL 60612
Fed Proc; 36(3):349 1977

The role of estrogen in the growth and spread of malig ıant melanoma has been debated for 40 years. Recently it has been shown that many human melanomas contain detectable estroge. ceptors. The objectives of this study were to ascertain if hamster melanoma has estrogen receptors and if this animal tumor model can be used to study hormonal influences on melanoma. Melanoma grown in female hamsters was assayed for estrogen receptor by incubating ultracentrifuged (100,000 x g) extracts with varying concentrations of ^{3}H-estradiol (.1-25nM). Control samples were incubated with 1000x excess of unlabelled estradiol to correct for nonspecific binding. Bound and free ^{3}H-estradiol were separated by the dextran-charcoal method. The binding was saturable, with Scatchard analysis giving a Kd of 5.5x10**-9M. Binding was abolished by heating the extract to 55 degrees C for 30 minutes. The tumor contains about 35 femtomoles of estrogen receptor per mg protein. The presence of estrogen receptor in the tumor is suggestive of a biologic role of estrogen in the growth of the tumor. To test this hypothesis estradiol benzoate, 100 ng per day, was given to female hamsters (n=10) subcutaneously in oil for 21 days after tumor passage. This resulted in a 50% increase (p less than .05) in tumor weight when compared to oil-injected control animals (n=10). We conclude that hamster melanoma contains estrogen receptor and that the tumor may be hormone dependent. (Author Abstract)

113. COLONIC NEOPLASMS. TISSUE ESTROGEN RECEPTOR AND CARCINOEMBRYONIC ANTIGEN.

McClendon JE, Appleby D, Claudon DB, Donegan WL, DeCosse JJ
Vince Lombardi Colon Clinic, Milwaukee County General Hosp., Milwaukee, WI
Arch Surg; 112(3):240-241 1977

Twenty-one patients with colonic neoplasms (19 adenocarcinoma and 2 with benign adenomatous polyps) were screened for the presence of estrogen receptor activity in tumor tissue; the findings were correlated with carcinoembryonic antigen (CEA) levels in the tumor and serum and with the assessment of a spectrum of tumor and patient characteristics. Estrogen receptor protein was found in 24% of the colonic neoplasms. The receptor activity was independent of age or sex, the state of differentiation or spread of the tumor, and the concentration of CEA in the tumor. Estrogen receptor activity in colon tumors probably reflects novel protein synthesis resulting from dedifferentiation. Measurement of tumor estrogen receptor protein and CEA may have discriminatory value in the patient with metastatic adenocarcinoma and an unknown primary neoplasm. (15 refs)

114. OESTROGEN RECEPTORS IN HAMSTER TUMOURS.

Steggles AW, King RJ
Imperial Cancer Res. Fund., London, England
Eur J Cancer; 8:323-334 1972

Stilbestrol-or estradiol -induced, estrogen-dependent primary hamster kidney tumors, estrogen-dependent transplant tumors and estrogen-independent transplant tumors were homogenized and centrifuged. Supernatants were sedimented on sucrose gradients after labeling with (6,7-3H)estradiol. Cytoplasmic estrogen receptors with sedimentation values of 8S and 4S, and a nuclear receptor with a value of 5S, were detected. No correlation was apparent between the receptor content of the different tumors and their estrogen responsiveness. The estradiol-receptor complex in tumor tissue was acid labile and estrogen specific. Studies of the 8S and 4S cytoplasmic receptors with protamine, N-ethyl maleimide and KCl suggested that these receptors were related and were similar to those found in rat uterine cytoplasm. In normal immature or ovariectomized hamster uterus, only the 8S estrogen receptor was seen. In intact normal hamster uterus, both the 4S and the 8S receptors were present two days post-estrus discharge.

115. ESTROGEN BINDING PROTEINS IN THE KIDNEYS OF HAMSTERS WITH ESTRADIOL INDUCED RENAL CARCINOMA (MEETING ABSTRACT).

Anderson NS, David Y, Fanestil DD
University of California, San Diego, Department of Med, La Jolla, Ca 92093
Fed Proc; 35(3):568 1976

Kidneys of normal Golden Syrian hamsters (NH), hamsters with renal tumors following chronic treatment with 17beta-estradiol (TH), and normal Sprague-Dawley rats (NR) were examined in vitro for the presence of cytoplasmic estrogen binding proteins (CEBP) and nuclear estrogen binding proteins (NEBP). Cytosols from whole kidney homogenates were incubated with 1.0 x 10**-9M ^{3}H-estradiol (^{3}H-E) for 1 hr both in the absence and in the presence of a 1000x excess of non-radioactive estradiol (E). Dextran coated charcoal was used to remove free steroid and values are reported as specific binding plus or minus standard error. The CEBP of NH bound 6.5 plus or minus 0.3 x 10**-15 moles/mg protein while the CEBP of TH bound 13.7 plus or minus 10**-15. The amount of CEBP in NR was found to be 15.8 plus or minus 1.1 x 10**-15 moles/mg protein. Tissue slices of the kidneys in each group were incubated at 25 degrees C for 1.5 hr with 1.0 x 10**-9M ^{3}H-E plus or minus 1000x E, tissue fractionated and the isolated nuclei examined for the presence of NEBP. The concentration of NEBP was 4.1 plus or minus 1.4 x 10**-14 moles/mg protein in NH, 3.6 plus or minus 1.3 x 10**-14 moles/mg protein in Th and 21.8 plus or minus 0.8 x 10**-14 in NR. Therefore, the kidneys of the species in which renal tumors are induced (NH) do not have more EBP than kidneys of NR which are not susceptible to the induction of renal tumors by estrogens. (Author Abstract)

116. ESTROGEN TARGET CELLS. ESTABLISHMENT OF A CELL LINE DERIVED FROM THE RAT PITUITARY TUMOR MTT/F4.

Sonnenschein C, Soto AM, Colofiore J, Farookhi R
Tufts Univ. Sch. Medicine, Cancer Res. Center, Boston, MA 02111
Exp Cell Res; 101(1):15-22 1976

A cell line, designated FPG, derived from the transplantable rat pituitary tumor MtT/F4 was established in long-term culture. The established cell line carried markers similar to those of the original source of material. Several

clones from this line were isolated. Saturable estrogen binding proteins of high affinity were characterized both in the cytosol and in the KCl nuclear extract of the MtT/F4 tumor growing in castrated animals. Clones C1 FPG and C5 EPG also showed the presence of estrogen receptors in their cytosol. "Empty" resident nuclear receptors in both cloned cell lines were also characterized. These nuclear receptors were apparently not translocated from the cytoplasm in an estrogen, temperature-dependent step. The dissociation constants for the estrogen receptor were essentially the same (10**-10M) in the cytoplasmic and nuclear compartments of both the tumor and the cell lines derived from it. The number of binding sites/cell in the two clones did not vary significantly; they have about 7000-10,000 sites/cell. The modal chromosome number of these cells was 61. The availability of cell cultures capable of reproducing systems present in whole animals should contribute to an understanding of the mechanism of action of estrogens.

117. THE COMPARATIVE SPECIFICITY OF THREE OESTRADIOL-BINDING PROTEINS: RAT ALPHA-FOETOPROTEIN, RAT LIVER 17BETA-HYDROXY STEROID DEHYDROGENASE AND ANTI-(OESTRADIOL-6-CARBOXYMETHYLOXIME - BOVINE SERUM ALBUMIN) ANTISERUM.

Laurent C, DeLauzon S, Cittanova N, Nunez E, Jayle MF
Laboratoire Associe 87 du CNRS, UER Biomedicale des Saints-Peres, 45 rue des Saints-Peres, 75270-Paris Cedex 06, France
Biochem J; 151(13):513-518 1975

A study was made of the specificity of 3-estradiol-binding proteins. Two of these proteins were naturally occurring (rat alpha-fetoprotein and rat liver microsomal 17beta-hydroxy steroid dehydrogenase); the third was an artifically induced model, anti-(estradiol-6-carboxymethyloxime-bovine serum albumin) gamma-globulins. A specific binding procedure for each protein model permitted a determination of its affinity for estradiol and 30 other steroids. The results obtained brought to light the different areas of the steroid molecule important for its recognition by each of the three proteins. The two naturally occurring proteins (alpha-fetoprotein and 17beta-hydroxy steroid dehydrogenase) recognized the edge of the steroid defined by C-4, C-6, C-8 and C-15. The gamma-globulins recognized the opposite edge. Diethylstilbestrol was only recognized by the two naturally occurring proteins.

118. THE MEASUREMENT OF THE MESSENGER RNA LEVELS FOR ALPHA FETOPROTEIN IN RAT LIVER AND IN HEPATOMAS (MEETING ABSTRACT).

Innis MA, Miller DL
Roche Inst of Mol Biol, Nutley, NJ 07110
Fed Proc; 36(3):818 1977

Rat alpha fetoprotein (AFP), an estrogen-binding protein secreted by fetal liver and many fast-growing hepatomas, is barely detectable in normal adult animals. As part of a study of the mechanisms controlling the expression of the AFP gene, we have compared the levels of AFP mRNA in normal liver and in hepatomas by RNA-DNA hybridization techniques. The AFP mRNA was isolated by immunoadsorption of AFP-synthesizing polysomes from Morris hepatoma 7777 using monospecific anti-AFP immunoglobulin. Translation of this mRNA fraction in nuclease-treated reticulocyte lysate produced a single protein which was identified as AFP by SDS gel electrophoresis and immunoadsorption. Complementary DNA to AFP mRNA was synthesized by avian myoblastosis virus RNA dependent DNA polymerase, was characterized by alkaline sucrose density gradient sedimentation and was used as a hybridization probe. We found that at the C0T value where 50% of the AFP cDNA probe hybridizes to hepatoma mRNA, less than 1% of the probe hybridizes to total mRNA from adult rat liver. This demonstrates the specificity of the cDNA probe, and implies that the amount of functional AFP mRNA is very low in adult liver. Thus it appears that the control of AFP synthesis in adult liver occurs at the level of mRNA synthesis or processing, rather than at the translational or secretory stage. (Author Abstract)

119. MOLECULAR WEIGHTS OF ESTROGEN AND ANDROGEN BINDING PROTEINS IN THE LIVER OF XENOPUS LAEVIS.

Bergink EW, Wittliff JL
Univ. Rochester Medical Center, Rochester, N.Y. 14642
Biochemistry (Washington); 14(14):3115-3121 1975

(3 H)Estradiol-17beta and (3 H)dihydrotestosterone binding proteins in the cytosol fraction of the liver of male and female Xenopus laevis were characterized by electrophoresis on polyacrylamide gels. These binding proteins, which were indistinguishable based on their mobilities in gels containing different acrylamide conc, migrated as single components with a molecular wt of 2.0 x 10^4. Separation of native or sodium dodecyl sulfate denaturated specific estrogen-binding components on dodecyl sulfate-free acrylamide gels gave similar results, i.e, a single species of molecular wt 2.0-2.5 x 10^4. The same molecular wt was also obtained when cytosol was prepared in the presence of either diisopropyl fluorophosphate or phenylmethanesulfonyl fluoride, both protease inhibitors. Evidence that the liver components binding (3 H)estradiol-17beta or (3 H)dihydrotestosterone were not plasma contaminants was provided by the observation that the plasma sex-steroid binding globulin of Xenopus had a different mobility when separated by polyacrylamide gel electrophoresis. These results, in conjunction with previous data, suggest that Xenopus liver has cytosolic components containing only estrogen-binding sites as well as other such components which may contain sites with affinities for both (3 H)estradiol-17beta and (3 H)dihydrotestosterone.

120. INTERACTIONS OF ESTROGEN AND ESTROGEN-PROTEIN COMPLEXES WITH XENOPUS LIVER NUCLEI (MEETING ABSTRACT).

Croall DE, Wittliff JL
Dept of Biochemistry, Univ of Rochester Medical Ctr, Rochester, NY 14642
Fed Proc; 36(3):816 1977

Previously we described the presence of specific estrogen binding components in Xenopus liver cytosol. In this study the association of (3H)estradiol-protein complexes with liver nuclei was demonstrated using a cell free system. Nuclear binding was maximal within 90 min at either 4 degrees or 22 degrees C. Nonspecific binding to nuclei was rapid (less than 2 min) and remained constant for 3 hr. The quantity of unbound ligand in preparations of estradiol-protein complexes did not alter the amount of steroid specifically retained by nuclei after 3 hr. Protein-bound steroid was recovered from nuclear sediments in an increasing fashion by elevating (KCl); 60% was extracted at 0.6 M. Nuclear suspensions were titrated with charged cytosol and revealed two types of interactions. One had a low capacity (**approx 2000 sites/cell) and a high affinity (Kd = 3 X 10**-10M) while the other had a higher capacity (**approx 8000 sites/cell) and a lower affinity (Kd = 2 X 10**-9M). These data suggest that the estrogen-binding protein complexes from Xenopus liver cytosol interact specifically with the nuclear compartment. This association may be essential for estrogen-induced synthesis of vitellogenin, the precursor for egg yolk proteins. (Author Abstract)

121. ESTROGEN AND PROGESTERONE RECEPTORS: METHODS FOR CHARACTERIZATION, QUANTIFICATION, AND PURIFICATION. (PP. 367-417)

Clark JH, Peck EJ, Schrader WT, O'Malley BW

Methods in Cancer Research. Busch H, ed. New York, Academic Press, vol. 12, 1976.

The interaction of the steroid hormones estrogen and progesterone with their respective target tissues depends on the binding of these steroids to specific macromolecules called receptors. The criteria for steroid receptors include finite binding capacity, high affinity for their respective hormone, tissue specificity, and the demonstration of a receptor-dependent hormonal response. Assays of steroid receptors must measure bound steroid and differentiate among free cytoplasmic receptors, Rc, occupied cytoplasmic receptors, RcS, and nuclear receptor-steroid complexes, RnS. Assay methods for cytosol receptors include equilibrium dialysis and adsorption techniques, the latter having the advantage because of their rapidity and ease of performance. Two different approaches have been used to purify receptor proteins from target tissues: (1) steroid affinity chromatography, which is the easier and more efficient approach; and (2) a more general method that uses the classical techniques of ion exchange, adsorption, and gel filtration chromatography. Other analytical and purification methods are based on receptor size and include gel filtration, sucrose-gradient ultracentrifugation, and gel electrophoresis. The assay of nuclear receptor-steroid complexes by KCl extraction techniques is complicated by a variety of factors that make strict quantitation difficult or impossible. The alternative procedure, ^{3}H-labeled steroid exchange, does not depend on extraction and has proved to be reasonably quantitative. In estrogen-independent tumors, which generally contain very low levels of estrogen receptor, withdrawal does not cause tumor arrest or regression, as is the case in estrogen-dependent tumors. This suggests that receptor-estrogen (RE) complexes may be involved either directly or indirectly in stimulating tumor growth. Since the RE complex is intimately involved in the stimulation and control of normal growth, the differences that might exist between normal and abnormal steroid receptors should be investigated. (120 refs)

122. COUMESTROL, NBD-NORHEXESTROL, AND DANSYL-NORHEXESTROL, FLUORESCENT PROBES OF ESTROGEN-BINDING PROTEINS.

Lee YJ, Notides AC, Tsay YG, Kende AS

Dept. Pharmacology and Toxicology, Univ. Rochester, Rochester, NY 14642

Biochemistry (Washington); 16(13):2896-2901 1977

The properties of coumestrol, dansyl-norhexestrol, and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD) norhexestrol as specific fluorescent probes of the estrogen receptor are reported. Coumestrol is a fluorescent estrogen with an extinction coefficient of 24,800 M**-1 cm**-2 at 345 nanom; at pH 7.5 it has an emission max at 438 nanom, a quantum yield of 0.20, and a fluorescence lifetime of 6 nanosec. A complex of coumestrol and bovine serum albumin (BSA) had an emission at 400 nanom and a quantum yield of 0.74. The dissociation constant of coumestrol was determined to be 1.4 (0.02) x 10**-7 M with a single binding site per molecule of albumin. Spectrofluorometric examination of the coumestrol-estrogen receptor complex showed a blue shift from 438 to 410 nanom and an increase in quantum yield. The spectroscopic properties and interactions of NBD-norhexestrol and dansyl-norhexestrol were also examined. The use of these compounds provides a new tool for the investigation of the properties of the estrogen receptor. (24 Refs)

123. SPECIFIC ESTROGEN RECEPTOR IN RAT AND BEEF THYMUS (MEETING ABSTRACT).

Grossman CJ, Nathan P

Depts of Physiology and Surgery, Univ of Cincinnati, Col of Med, Cincinnati, OH 45219

Fed Proc; 36(3):1236 1977

Rat thymus weights from adult males increase upon castration and decrease when the animals are treated with estrogen. This response to the steroid suggested that the thymus might contain specific estrogen receptor. Thymus from male rats aged 1-2 mos or from 1-2 yr old steers or heifers was analyzed. The tissue was homogenized in 0.01M Tris, 1mM EDTA and centrifuged at 120,000xg for 30 mins. The concentration of specific estrogen receptor was determined in the supernatant cytosol by Scatchard plot analysis; specificity of binding was established by competition studies. From Scatchard plot analysis the association constant, KA, for beef thymus was shown to be 1.719 plus or minus .09 x 10^9 M**-1 (N=6) and for rat thymus was 6.618 plus or minus .75 x 10^9 M**-1 (N=6). The receptor concentration for beef thymus was 0.33 plus or minus .03 (N=5) pmoles/g tissue and for rat thymus was 0.31 plus or minus .02 (N=6) pmoles/g tissue wet weight. In terms of cytosol protein the concentration of receptor for beef thymus was 6.86 plus or minus .47 (N=5) fmoles/mg protein and for rat was 7.18 plus or minus .61 (N=6) fmoles/mg protein. With estrogen binding set at 100% competition studies showed dihydrotestosterone binding was less than 3% while testosterone, progesterone, cortisol and triamcinolone did not significantly compete with estrogen for receptor binding sites. From these preliminary results it is concluded that homogenates of both beef and rat thymus contain high affinity, specific estrogen receptors. (Author Abstract)

124. BINDING OF ESTROGENS TO SERUM PROTEIN OF RATS TREATED WITH HEPATOCARCINOGENIC COMPOUNDS.

Nunez E, Bennassayag C, Engelmann F, Vallette G, Hurst L, Jayle MF

Laboratoire de Biochimie, U.E.R. Biomedicale des Saint-Peres, Paris, France

Biomedicine; 18(6):514-520 1973

The binding of a series of steroid hormones by the serum of ascitic fluid of Wistar rats treated with hepatocarcinogenic compounds paradimethylaminoazobenzene or 2-acetamidofluorene was studied. The sera of animals with histologically identified hepatomas producing alpha1-fetoprotein displayed a significantly greater affinity for estradiol and estrone than the sera of the other animals. There are three distinct binding sites for estradiol in the sera of the ascitic fluid of some hepatoma bearing rats: the first is present in low concentration but displays a very high affinity; the second is more concentrated and has a lower affinity; and the third shows a very low affinity probably corresponding to the albumin. Using autoradioimmunoelectrophoresis, the estradiol binding proteins were shown to be a lipoprotein esterase, while the second is located near the albumin and the third corresponds to alpha1-fetoprotein.

D. The Characterization and Role of Androgen Receptors and Androgen Binding Proteins

125. THE MEASUREMENT OF ANDROGEN RECEPTORS IN HUMAN PROSTATIC TISSUE UTILIZING SUCROSE DENSITY CENTRIFUGATION AND A PROTAMINE PRECIPITATION ASSAY.

Menon M, Tananis CE, McLoughlin MG, Lippman ME, Walsh PC
James Buchanan Brady Urological Inst., Johns Hopkins Hosp., Baltimore, MD 21205
J Urol; 117(3):309-312 1977

A technique for the measurement of androgen receptors in the cytosol and crude nuclear extracts of human prostatic tissue has been developed. The method uses sucrose density gradient centrifugation and a protamine precipitation assay. These studies demonstrated the presence of a high affinity, heat labile protein that binds preferentially to dihydrotestosterone and is present only in androgen target tissues. Seventy-two assays of prostatic tissue from 15 patients indicated that the sucrose density gradient centrifugation was more sensitive than the protamine assay in the identification of androgen receptors. These findings indicate that the technique may be a reliable measurement of androgen receptors in prostatic carcinoma. (20 refs)

126. ANDROGEN BINDING PROTEINS IN HUMAN PROSTATE.

Jung-Testas I, Mercier-Bondard C, Robel P
ER 125 CNRS, Unite de Recherches sur le Metabolisme Moleculaire, Hopital de Bicetre, 78, rue du General Leclerc, F 94270 Bicetre, France
Ann Endocrinol (Paris); 37(2):97-98 1976

Two high affinity saturable binding components were observed in the cytosol from hyperplastic human prostates centrifuged in a glycerol gradient (sedimentation coefficient of 8-10S and 4-5S). Steroid binding experiments showed one to be the androgen-receptor protein and the other, the sex steroid binding plasma protein (SBP). The cytosol receptor concentration was 5,000 sites/cell. The SBP concentration, measured by equilibrium dialysis, was 8,000 sites/cell. Prostate samples from hyperplastic and normal individuals were subjected to constant flow organ culture in the presence of H3-testosterone and increasing amounts of human serum albumin (HAS) and/or SBP. The decrease of albumin binding to testosterone was directly proportional to the decrease in the fraction of unbound testosterone in the perfusion fluid; the relative proportions of testosterone metabolites were not modified. However, SBP decreased the penetration of testosterone and increased the proportion of unmetabolized testosterone, suggesting the SBP-testosterone complex may be specifically associated with the prostate cells. (no refs)

127. ANDROGEN BINDING AND METABOLISM IN THE HUMAN PROSTATE.

Attramadal A, Tveter KJ, Weddington SC, Djoseland O, Naess O, Hansson V, Torgersen O
Rikshopitalet, Oslo, Norway
Vitam Horm; 33:247-264 1975

Androgen binding and metabolism in the human prostate are reviewed, and results with neoplastic tissue are compared to those with nonneoplastic tissue. Both types of prostatic tissue contain at least two soluble androgen-binding proteins, as demonstrated by polyacrylamide gel electrophoresis, sucrose gradient analysis, Sephadex G-200 gel filtration, and Sephadex G-200 chromatography. One of these binding proteins has characteristics similar to those of the intracellular receptors in accessory sex organs of the male rat, while the other has properties similar to those of testosterone-binding globulin. No differences in physicochemical properties between the receptors from neoplastic and nonneoplastic tissues were demonstrated. Human prostatic tissue contains oxidative and reductive enzymes that act on testosterone. Reduction to 5a-dihydrotestosterone is carried on more rapidly in hyperplastic tissue than in prostatic cancer tissue. Further studies of receptor concentrations and of 5a-reductase activity in prostatic tissue might be of use in selecting patients for endocrine therapy.

128. PRODUCTION OF TESTOSTERONE BY PROSTATE AND OTHER PERIPHERAL TISSUES IN MAN.

Lipsett MB
Case Western Reserve Univ. Sch. Medicine, Cleveland, OH
Vitam Horm; 33:209-221 1975

Methods for studying androgens and proandrogens in various body fluids and tissues are reviewed and the origins of the major androgens are considered. The production of steroids in the blood can be estimated as the product of the metabolic clearance rate (MCR) and the plasma steroid concentration. The MCR is defined as the volume of blood or plasma irreversibly cleared of steroid per unit time. The determination of MCR involves the infusion of two steroids with different labels and measuring the ratio of isotopes in the product. The technique takes about 2 hr with the subjects in a supine position. Tissue extraction is determined both by the rate of steroid transport from plasma and by the rate of enzymic metabolism in the cells. The transport of steroid from plasma is in turn influenced by steroid binding to plasma proteins. It may be difficult to ascertain whether plasma protein binding or intracellular metabolism is rate limiting in overall tissue extraction, especially since these two processes can rarely be studied simultaneously. Testosterone binds with great affinity to the plasma protein, testosterone-estradiol binding globulin (TEBG), and to other plasma proteins but with less affinity than to TEBG. In normal men, more than 95% of the testosterone production is a result of Leydig cell secretion. In orchiectomized men, however, most of the testosterone in blood is produced by peripheral metabolism of steroids secreted by the adrenal cortex. About 50% of dihydrotestosterone is derived from plasma testosterone and an additional 25% from androstenedione. The testis also secretes some dihydrotestosterone. Dehydroepiandrosterone is secreted primarily by the adrenal cortex, both free and as the sulfate, and small amounts are secreted by the ovary and by the testis. Plasma concentrations of androstenediol are twice as high in men as in women; it is secreted by the testis and the ovary but not by the adrenal cortex. In an orchiectomized man, therefore, the origin of androstenediol is almost entirely from the peripheral metabolism of dehydroepiandrosterone. Androstanediol originates from plasma testosterone and from androstenedione. All of these steroids have demonstrated biological androgenicity in the rodent bioassay system but may have low binding affinity for cytosol receptor. This apparent discrepancy is due in part to peripheral transformations of the steroid and to the quantitatively modified transformations occurring in target tissues.

129. ANDROGEN RECEPTORS AND ANDROGEN-DEPENDENT INITIATION OF PROTEIN SYNTHESIS IN THE PROSTATE.

Liao S, Tymoczko JL, Castaneda E, Liang T
Ben May Lab. Cancer Res., Univ. Chicago, Chicago, IL
Vitam Horm; 33:297-317 1975

Recent studies relating to elucidation of the receptor mechanism involved in the action of 5a-dihydrotestosterone (DHT) are reviewed. Binding proteins have been demonstrated in both cytosol and microsomal fractions of the prostate. DHT-receptor complexes sediment at 7-12 S and 3-5

S units, the larger units being converted to the smaller by incubation at 20-30 C. Studies of structural requirements for binding indicate that the bulkiness and flatness of the steroid molecule play a more important role in receptor-binding than the detailed electronic structure or atoms of the steroid nucleus. A simple quantitative and qualitative assay of the steroid-receptor complex makes use of the interaction with antisteroid antibodies. Various metal ions have differing effects on the sedimentation properties of the complex. Both ATP and GTP can interact with and stabilize the DHT-receptor complex, and binding to some but not all polynucleotides has been demonstrated. DHT-receptor complex forms a 60-80 S complex with ribonucleoprotein from prostate, but not from liver. Purified prostate cell nuclei can selectively retain the DHT-receptor complex. The DHT-dependent translocation of the cytoplasmic receptor protein to the prostate cell nuclei is temperature-dependent. Heating or proteolytic treatment of nuclei can result in the loss of specific binding of the DHR-receptor complex. Prostate ribosomes from androgen-treated rats have higher protein synthesizing ability than those from control castrates, an effect attributed to the androgen-dependent increase in mRNA content. Experimental evidence that steroid hormones and their receptors act primarily at the level of gene transcription is cited. The role of the steroid-receptor complex may involve action at the level of processing and utilization of RNA in addition to the primary function of regulating RNA synthesis.

130. MEASUREMENT OF ANDROGEN RECEPTORS IN HUMAN PROSTATIC TISSUE: METHODOLOGICAL CONSIDERATIONS.

Walsh PC, McLoughlin MG, Menon M, Tananis C
Prostatic Disease Proceedings of an American-European Symposium Held in Vienna, 3-5 November 1975. Physicians Associated for Continuing Education Vienna Austria, Progress in Clinical and Biological Research, 1976.

Attempts to quantitate androgen receptors in human prostatic tissue are reviewed. Initial studies of the binding of dihydrotestosterone suggest that cytoplasmic receptor proteins is bound mainly to testosterone-estradiol-binding globulin (TeBG), a serum protein. Purification of prostatic cystosol by ammonium sulfate fractionation and diethylaminoethyl-cellulose chromatography of high-salt nuclear extracts did not eliminate the binding to TeBG. However, high-affinity binding proteins were demonstrated in the cytosol and nuclear extracts of benign hyperplastic tissue by sucrose density-gradient centrifugation and by the protamine sulfate assay for specific receptor sites. The relevance of these findings for the therapy of prostatic carcinomas is discussed. (32 refs)

131. PROTEIN BOUND ANDROGENS IN THE HYPERPLASTIC HUMAN PROSTATE: METHODOLOGICAL ASPECTS.

Dube JY, Tremblay RR, Coulonval L
Laboratoire d'Endocrinologie et Metabolisme, le Centre Hospitaliere de l'Universite Laval, 2705, Boul Laurier Ste-Foy, Quebec, GIV 4G2 Canada
Union Med Can; 104(8):1224-1228 1975

Three different methods were used to analyze the cytosol of the human hyperplastic prostate for proteins capable of binding to the androgens but different from the plasma testosterone-estradiol binding globulin (TeBG). Prostatic tissue obtained by transvesical prostatectomy was studied by gel filtration on Sephadex G-25 columns, a polyacrylamide or agarose gel electrophoresis. Gel filtration did not determine the androgen receptors specifically because it detected binding to all high androgen affinity proteins. The two other methods detect only the binding to the plasma TeBG contaminating the tissue. Thus, these methods cannot be used as such to determine androgen receptors in the human prostate. Once a way is found to completely and specifically inhibit plasma TeBG, the gel filtration or charcoal method of detection of androgen-binding proteins will be useful. (18 refs)

132. ANDROGEN RECEPTORS IN HUMAN PROSTATIC TISSUE: A REVIEW.

Menon M, Tananis CE, McLoughlin MG, Walsh PC
James Buchanan Brady Urological Inst., Johns Hopkins Hosp. Baltimore, MD 21205
Cancer Treat Rep; 61(2):265-271 1977

Methods for the measurement of androgen receptors in the human prostate are presented and reviewed. While the androgen receptor in the rat ventral prostate has been studied extensively and is easily characterized on sucrose gradients the characterization of the human androgen receptor has been complicated by the presence of testosterone-estradiol binding globulin (TeBG) in the serum. TeBG has a sedimentation coefficient of 4.5S and binds dihydrotestosterone (DHT) with high affinity. If the tissue is contaminated by as little as 4% serum, the characterization of the androgen receptor is rendered impossible by the presence of TeBG. The characterization of androgen receptor proteins is based on their ability to bind to radioactive ligands of high specific activity. Four criteria must be satisfied to specifically quantitate this binding: hormonal specificity, tissue specificity, high affinity, and saturability. Gel filtration, density gradient centrifugation, ion-exchange chromatography, electrophoresis, and protamine precipitation assays are reviewed as the methods most often used in these investigations. It is concluded that sucrose density gradient centrifugation, ion-exchange chromatography and the protamine precipitation assay are the most specific methods for the identification of androgen receptors. (26 Refs)

133. STEROID HORMONE RECEPTOR CHARACTERIZATION OF THE DUNNING R-3327 PROSTATIC ADENOCARCINOMA (MEETING ABSTRACT).

Markland FS, Lee L
LACUSC Cancer Center, Los Angeles, CA 90033
Proc Am Assoc Cancer Res; 18:151 1977

In view of the reported hormonal dependence of the Dunning prostatic adenocarcinoma, work was initiated to identify and characterize the steroid hormone receptors in the tumor. Tumors were removed 7-9 months after transplantation and stored in liquid N2. Cytosol was prepared and incubated with tritiated hormone (with or without excess radioinert hormone) for 3 hours at 0-3 degrees C. Binding was analyzed by sucrose density gradient centrifugation in low salt (SG). Specific binding was found in both the 8S and 4S regions after incubation with ^{3}H-methyltrienolone (^{3}H-R1881,Roussel Uclaf, Paris). Specific binding in the 8S region was inhibited by 100 fold excess R1881. Estradiol-17beta (E2) and a synthetic progestin (R5020, Roussel Uclaf) inhibited binding 75% and 15-20%, respectively. Dexamethasone (DXM) had no effect on R1881 binding. Scatchard analysis indicated a single class of high affinity binding sites with a Kd of 5x10**-9 M and a total binding capacity of 50-70 fmoles/mg cytosol protein. Plasma from male Copenhagen rats contained no suppressible R1881 binding. The tumor also contained a specific ^{3}H-E2 receptor which sedimented in the 8S region on SG. This binding was completely inhibited by E2 or DES. R1881, R5020 or DXM did not inhibit binding. No specific progestin or glucocorticoid binding was observed in the 8S region on SG. The presence of steroid hormone receptors helps explain the hormonal sensitivity of this tumor. (Author Abstract)

134. ANDROGEN BINDING IN THE TESTIS: IN VITRO PRODUCTION OF ANDROGEN BINDING PROTEIN (ABP) BY SERTOLI: CELL CULTURES AND MEASUREMENT OF NUCLEAR BOUND ANDROGEN BY A NUCLEAR EXCHANGE ASSAY. (PP. 293-309)

Sanborn BM, Elkington JS, Steinberger A, Steinberger E

Hormonal Regulation of Spermatogenesis. French FS, Hansson V, Ritzen EM, Nayfeh SN, ed. New York, Plenum Press, Current Topics in Molecular Endocrinology, vol. 2, 1975.

Studies on androgen binding in the testis and the recognition of two components in the binding are reported. The first, androgen binding protein (ABP), was produced by Sertoli cells in culture for at least 9 days in the absence of exogenously added hormones. Follicle stimulating hormone (10-100 microg/ml) markedly enhanced the secretion of ABP. Methyl-isobutylxanthine had a potentiating effect after 7 days of treatment. A nuclear exchange assay was developed to study the second component. In the assay, activated steroid-receptor complexes migrated to the nucleus and bound to acceptor sites. The nuclei were then isolated and labeled hormone was exchanged for unlabeled hormone under stable conditions. Competition for exchange activity using H3-dihydrotestosterone was significant for a 500-fold excess of testosterone, dihydrotestosterone, progesterone, and cryproterone acetate. The exchange activity was increased 2-10 fold by prior in-vivo or in vitro-treatment with testosterone. Significant exchange activity was found in long-term hypophysectomized adult and immature animals and in tubule and germ cell fractions. In isolated germ cell fractions, the highest concentration of exchange activity was associated with the most mature elements. These data suggest that androgen exchange activity may exist in both Sertoli cell and germ cell fractions and suggest that the mechanism of action of androgens in the testis is complex. (35 refs)

135. AN APPROACH TO THE MECHANISM OF ANDROGEN ACTION.

Baulieu EE

Host Environment Interactions in the Etiology of Cancer in Man; Proceedings of a Meeting Held at Primosten, Yugoslavia, 27 August-2 September 1972. International Agency for Research on Cancer and John E. Fogarty International Center of the Natl Insts of Health USA. (Lyon, France):143-147 1973

Reports on the mechanism of androgen metabolism and transport are reviewed. One study on testosterone metabolism showed a "17b-hydroxy pathway", along which there are the active metabolites androstanolone, dihydro- testosterone, 3a androstanediol, and 3b-androstanediol. Substances that are involved in testosterone transport include corticosteroid-binding globulin and a sex steroid-binding plasma protein. A high-affinity, low- capacity, androstanolone-binding protein has been observed in ventral prostate cytosol; the sedimentation coefficient in low ionic strength Tris buffer and its reversible dissociation by potassium chloride into 4-5 S subunits make it very similar to other steroid receptors. Other studies suggest that testosterone acts through different metabolic patterns in different target tissues.

136. STUDIES ON THE UPTAKE AND BINDING OF 5A-ANDROSTANE-3A,17A-DIOL BY CANINE PROSTATIC NUCLEI.

Evans CR, Pierrepoint CG

Tenovus Inst. Cancer Res., Welsh Natl. Sch. Medicine, Heath, Cardiff, CF4 4XX, England

J Endocrinol; 70(1):31-37 1976

The transfer of radioactivity to the nucleus was examined in male dogs following the incubation of whole canine prostatic tissue with either tritiated testosterone or 5alpha-androstane-3alpha,17alpha-diol, with subsequent characterization of the bound radioactive hormone. The nuclei were isolated; the protein-bound radioactivity was shown to be associated with 5alphaandrostane-3alpha,17alpha-diol. The molecular wt of the binding protein was between 80,000 and 90,000. The formation and translocation of this steroid to the nucleus supports previous evidence that it has an active part to play in prostatic function in the dog. The formation from testosterone of another tritiated steroid, possibly 5alpha-dihydrotestosterone, was also demonstrated although this product did not enter the nucleus and was apparently bound to the nuclear membrane.

137. ANDROGEN BINDING PROTEIN IN THE RAT PROSTATE: METHODOLOGICAL PROBLEMS AND REGULATION.

Blondeau JP, Corpechot C, Robel P

ER 125 CNRS, Unite de Recherches sur le Metabolisme Moleculaire, Hopital de Bicetre, 78, Rue du General Leclerc, F 94270 Bicetre, France

Ann Endocrinol (Paris); 37(2):95-96 1976

Selective and quantitative protamine sulfate precipitation was used for the study of equilibrium-binding constants of the ventral prostate of the rat. Binding constants of 17-beta-hydroxy-5-alpha-androstan-3-one (dihydrotestosterone) were measured in adult male rats (250-300 g; number not specified) which had been castrated 24 hr earlier. The association constant of dihydrotestosterone was 1 x 10^9 liter/mole, and the binding sites numbered 11,500/cell. The affinity of testosterone was slightly lower than that of dihydrotestosterone, whereas certain synthetic androgens have a higher affinity for the receptor in accordance with their biological activity. Estradiol, progesterone, and various antiandrogens displaced dihydrotestosterone from its receptor site, but no estradiol-specific nor progesterone-specific receptors were observed. In the intact or testosterone-treated rat, cytosolic and nuclear binding sites can be measured by radioimmunoassay. Binding sites number 65,000/cell, with most in the nuclei (82%). Following castration, the nuclear hormone-receptor complexes disappear with a half-life of 3 hr. The cytosol receptor decreases between the 1st and 4th day after castration, then increases spontaneously. Testosterone seems to inhibit degradation and to stimulate the synthesis of its receptor. Autoradiography indicates that the receptor is present only in the epithelial cells. (no refs)

138. INTERACTION OF SPIRONOLACTONE AND DIGITALIS WITH THE 5ALPHA-DIHYDROTESTERONE (DHT) RECEPTOR OF RAT VENTRAL PROSTATE.

Pita JC, Lippman ME, Thompson EB, Loriaux DL

Natl. Inst. Child Health Human Development, Natl. Inst. Health, Bethesda, Md. 20014

Endocrinology; 97(6):1521-1527 1975

The possible interactions of spironolactone, digitoxin, and digitoxigenin with the 17-beta-hydroxy-5alpha-androstan-3-one (5-alpha-dihydrotestosterone, DHT) receptor of rat ventral prostate were studied. The competitive receptor assay involved precipitation of the 105,000 x g supernatant of the homogenized Holtzman rat prostatic tissue with protamine sulfate, removal of the unprecipitated cytosol, and incubation of the precipitate in the presence of the appropriate ^{3} HDHT steroid solution at 0 C for 18 hr. The Kd (dissociation constant) for DHT was between 1.9 and 4.0 x 10**-9 M and the binding capacity was 0.21 pmol/mg protein. Spironolactone interfered with the binding of DHT to the precipitated cytosol and displayed an estimated Kd of 1.3-4.6 x 10**-8 M. Several digitalis preparations were similarly studied. Digitoxin and digitoxigenin also interfered with the

binding of ³ HDHT with an estimated Kd of 0.8-3.6 x 10**-8 M. Digoxin interacted less strongly and its estimated Kd was 10**-6 M. These results suggest an interaction, or interference, of spironolactone and digitalis with the DHT receptor in prostate cytosol and may help explain some of their antiandrogenic actions in the rat and in man.

139. DIRECT EVIDENCE ON INCORPORATION OF THE RECEPTOR INTO THE NUCLEI OF RAT VENTRAL PROSTATE IN THE FORM OF THE COMPLEX WITH DIHYDROTESTOSTERONE.

Nozu K, Yitoh H, Tamaoki BI
Kyoto Res. Lab., Kakenyaku Kako Co. Ltd., Yamashina, Higashiyama-ku, Kyoto 607, Japan
Endocrinol Jpn; 22(6):537-548 1975

The cytosol 9S receptor, labeled with ¹³¹ iodine (¹³¹ I) retained the activity of forming a complex with ³ H-dihydrotestosterone, when examined by a sucrose density gradient centrifugation. When the receptor was incubated with isolated prostatic nuclei in the presence of ³ H-dihydrotestosterone, it was directly incorporated into the nuclei in a form of the complex bound to ³ H-dihydrotestosterone. The incorporation of ¹³¹ I-labeled receptor**-3 H-dihydrotestosterone complex into the nuclei increased along with the raised temperature of incubation, whereas association of the complex with the chromatin reached maximum at 35 C and then decreased gradually beyond this temperature. In the time course study, either the incorporation of the complex into the nuclei or the association with the chromatin reached the maximal levels within 10 min and leveled off up to 60 min. ¹³¹ I-labeled serum protein was far less efficiently incorporated into the nuclei than the radioiodinated 9S receptor; association of the serum protein with the chromatin was limited to a very little extent. The ¹³¹ I-labeled 9S receptor-dihydrotestosterone complex associated with the chromatin was preferentially distributed into the non-histone protein as well as the DNA itself of the chromatin. The radioactivity lost by dehistonization of the chromatin was almost negligible. The cytosol 9S receptor fraction bound to ³ H-dihydrotestosterone was purified about 100 fold by the two consecutive column chromatographies. The partially purified receptor fraction which was labeled with radioiodine incorporated mostly into non-histone protein and DNA fractions. Apparently the cytosol receptor is required not only for transport of the steroid hormone to the nucleus but also for interaction of the hormone-receptor complex with the chromatin.

140. 5A-DIHYDROTESTOSTERONE-BINDING PROTEINS AND ANDROGEN SENSITIVITY IN PROSTATIC CANCERS OF COPENHAGEN RATS.

Voigt W, Feldman M, Dunning WF
Univ. Miami Sch. Medicine, Miami, FL 33136
Cancer Res; 35(7):1840-1846 1975

The establishment of a non-androgen-responsive line (R-3327-A) of prostatic cancers derived from the R-3327 line (Copenhagen rats) was reported. Tumors of the new line have a growth rate equal to 10x that of its parent series; no differences in growth rate were observed for females or males (castrated or non-castrated). The distribution of radioactivity 1 hr after inj of tritiated testosterone in the R-3327-A line was 17-beta-hydroxy-5-alpha-androstan-3-one (DHT;40%), 5-alpha-androstan-3-alpha, 17-beta-diol (6.5%), and 5-alpha-androstan-3,17-dione (6.7%) in prostatic tissue and 8.8% 3.1%, and 3.6%, resp, for the same metabolites in tumor tissue. A 3.5s protein, found to bind DHT and estradiol in R-3327, was not observed in the new tumor line, suggesting a lack of androgen binding protein for this insensitive strain. The synthesis of binding protein is thought to be regulated by circulating androgen levels. R-3327-A tumors are squamous cell carcinomas, while those of R-3327 are adenocarcinomas. An assay for androgen receptors in human prostatic cancer may become a diagnostic aid for choosing an appropriate mode of therapy.

141. MECHANISM OF ANDROGEN ACTION AT THE CELLULAR LEVEL.

Sluyser M, Kassenaar AA
The Netherlands Cancer Inst., Sarphatistraat 108, Amsterdam, The Netherlands
Pharmacol Ther Part B; 1(2):179-188 1975

The major steps in the mechanism of action of androgens are reviewed. In both rats and humans, injected testosterone is rapidly taken up by the prostate and at least 90% is then converted to androstandiol, androsterone, and 5a-dihydrotestosterone (DHT). Prostatic nuclei reduce testosterone to DHT, and in the cytoplasm both DHT and androstandiol are formed. Receptors for DHT have been detected in the nuclei and cytosol of rat prostates, and androgen receptors have been identified in the accessory sexual glands and certain experimental androgen-dependent tumors in several species. No receptors have been found in most types of skeletal muscle. In one study, samples of hypertrophied human prostate gland were shown to contain a large amount of DHT, but in another DHT receptors were not detected in all samples of hypertrophied human prostate studied. The hypothalamus appears to be a target tissue for androgens. In the absence of cytosol, purified prostatic nuclei alone were unable to bind or retain DHT to any appreciable extent, suggesting that cytoplasmic DHT receptors are implicated in the nuclear binding of DHT. DHT receptors appear to bind specifically to DNA obtained from the prostate by the detergent method. DHT receptors stimulate RNA polymerase activity in the nuclei of androgen-sensitive cells; they do not enhance the cytoplasmic form of DNA polymerase. It is unclear how the binding of androgen-receptor complexes to nuclear chromatin causes the nuclei to produce increased amounts of RNA. Testosterone mainly induces the synthesis of ribosomal RNA and also increases the activity of seminal-vesicular phosphofructokinase. Androgens control the expression of liver N'-methylnicrotinomide oxidase and affect mitochondrial protein synthesis in the rat prostate.

142. ASSAY OF ANDROGEN BINDING SITES BY EXCHANGE WITH METHYLTRIENOLONE (R 1881).

Bonne C, Raynaud J
Centre de Recherches Roussel-Uclaf, 93230 Romainville, France
Steroids; 27(4):497-507 1976

Methyltrienolone (R 1881 - 17beta-hydroxy-17alpha-methyl-estra-4,9,11-trien-3-one) binds specifically to androgen receptor in rat prostate cytosol where, unlike androstanolone, it is not metabolized. By exchanging bound endogenous hormone in rat prostate cytosol with labelled R 1881, it is possible to measure total (free and occupied) binding sites. This assay method has also been applied to the measurement of androgen receptor sites in human benign prostatic hypertrophy where R 1881 has the added advantage of not being bound by any contaminating plasma protein (sex hormone binding protein). (Author Abstract)

143. CHARACTERIZATION OF THE ANDROGEN RECEPTOR FROM A SYRIAN HAMSTER DUCTUS DEFERENS TUMOR CELL LINE (DDT1).

Norris JS, Kohler PO
Baylor Coll. Medicine, Houston, TX 77025
Science; 192(4242):898-900 1976

The complex cytoplasmic androgen receptor protein, which binds ³ H-labeled 5,a-dihydrotestosterone, of the

hamster ductus deferens cloned tumor cell line (DDT1) is characterized. Diethylaminoethyl (DEAE) chromatography of cytosol from these cells yielded two major receptor peaks of activity. Identification of this steroid binding protein as a cytoplasmic receptor was confirmed by salt dissociation on sucrose gradients, stability of the hormone-receptor complex at 0 C, and the retention patterns on phosphocellulose and DEAE cellulose. The authors propose that the cytoplasmic form of the receptor is a 6S dimer, consisting of an A and B monomer, which moves into the nucleus and is bound to the chromatin acceptor site by the B subunit. The A subunit then dissociates from the B monomer and searches along adjacent chromatin regions until it locates and binds to a specific regulatory site on naked DNA. This model would explain the presence of subunits with different functions, but both bind steroid hormone.

144. FSH STIMULATION OF TESTICULAR ANDROGEN BINDING PROTEIN (ABP): COMPARISON OF ABP RESPONSE AND OVARIAN AUGMENTATION.

Hansson V, Weddington SC, Petrusz P, Ritzen EM, Nayfeh SN, French FS

Rikshospitalet, Oslo, Norway

Endocrinology; 97(2):469-473 1975

The dose response relation of stimulation by follicle-stimulating hormone (FSH) was examined, and the androgen binding protein-stimulating activities of several FSH preparations were compared with the ovarian wt augmenting activities. Sprague-Dawley rats in groups of 2 were injected for 1 to 8 days with 500 microg/day FSH-Pl or with the vehicle only (0.1% bovine serum albumin in saline). There was no binding protein response 1 hr after injection, in the caput epididymidis. Androgen binding protein was present after 24 hr, reaching a plateau at 3 to 4 days. No binding protein was detected in controls. Hypophysectomized rats in groups of 4 were injected with 31.25-500 microg FSH-Pl/day. The log dose response appeared linear in the range of 60-250 microg/day. Measurable stimulation of androgen binding protein was obtained with the lowest dose used. The data indicate that androgen binding protein production in response to various FSH preparations follows a dose-response relationship.

145. EVIDENCE FOR A SPECIFIC DIHYDROTESTOSTERONE-BINDING CYTOSOL RECEPTOR IN THE HUMAN PROSTATE.

Geller J, Cantor T, Albert J

Mercey Hosp. and Medical Center, San Diego, Calif. 92103

J Clin Endocrinol Metab; 41(5):854-862 1975

A technique for the demonstration of a human steroid and organ-specific prostate cytosol receptor is described. Tissue from 17 patients with benign prostatic hypertrophy was incubated in Eagle's medium with ^{3}H-testosterone (.06 nM) following preincubation with an antiandrogen. The minces were homogenized and the cytosol fraction obtained. The cytosol was then fractionated on a duo-gel column of G-50 Sephadex over Bio-gel A 1.5m and fractions counted for ^{3}H, assayed for protein and plotted graphically. Of 3 main peaks seen, only the second peak (approximately 150,000 molecular wt), which contained predominantly dihydrotestosterone (DHT), was significantly and reproducibly inhibited by 2.1 microM cyproterone acetate (Cyp A) preincubation. This inhibition was considered a specific indicator for receptor since Cyp A at microM had little effect (less than 10%) on ^{3}H-DHT binding to plasma. Similar results were noted for other antiandrogens tested (MK 316, medrogestone, megestrol acetate, and flutamide), but cortisol and etiocholanolone had no effects on ^{3}H-5alpha DHT binding to prostate cytosol. Steroid-protein peaks for fractionated human thyroid, muscle, and spleen cytosol were not inhibited by Cyp A. Fractionated kidney cytosol contained a ^{3}H-T-binding protein peak which was significantly decreased by Cyp A. Pronase incubation and heating at 50 C for 30 min resulted in either a significant decrease or complete loss of receptor. The results give strong evidence for the existence of a high-affinity, low-capacity binding protein (receptor) in human prostate cytosol distinct from plasma testosterone-estradiol-binding globulin. It is concluded that gel filtration analysis of ^{3}H-cytosol derived from human prostate minces pre-incubated with and without Cyp A, provides a relatively rapid technique for the demonstration of cytosol receptor in approximately 80% of prostates from patients with benign prostatic hypertrophy.

146. ACTIVE FORMS AND BIODYNAMICS OF THE ANDROGEN-RECEPTOR IN VARIOUS TARGET TISSUES. (PP. 139-151)

Liao S, Hang SC, Tymoczko JL, Liang T

Steroid Hormone Action and Cancer. Menon KM, Reel JR, ed. New York, Plenum Press, Current Topics in Molecular Endocrinology, vol. 4, 1976.

The biodynamics and active forms of the androgen receptor are described. In tissues of aging animals with the insensitivity found in autonomous tumors or testicular feminization, androgen insensitivity may be related to low receptor content, the qualitative changes in the active forms of the androgen-receptor complexes may be responsible in some cases. Some androgens bind to receptor proteins as if they are enveloped by the protein; the interaction may cause the transformation of the receptor protein to a form capable of functioning at the target site. It is concluded that no specific electronic structure or functional group is necessary for androgen action and receptor binding in the target cell aside from the gross geometric structure. (97 refs)

147. SIMILARITIES BETWEEN 5A-DIHYDROTESTOSTERONE-RECEPTOR COMPLEXES FROM HUMAN AND RAT PROSTATIC TISSUE: EFFECTS ON RNA POLYMERASE ACTIVITY.

Davies P, Griffiths K

Welsh Natl. Sch. Medicine, Heath, Cardiff, CF4 4XX, U.K.

Mol Cell Endocrinol; 3:143-164 1975

The role of steroid-receptor complexes in regulating RNA synthesis in Sprague-Dawley rat ventral prostate tissue and human hypertrophic prostate, and the ability of these complexes, labeled with ^{3}H5-dihydrotestosterone (^{3}HDHT), to transfer radioactivity into nuclear preparations was investigated. In addition, the stimulation of RNA polymerase activity of isolated nuclei was studied. Protein macromolecules specifically binding ^{3}HDHT were identified in cytosol and in nuclei prepared from human benign hypertrophic prostate. These macromolecules had similar properties to receptor proteins from other androgen-dependent tissues. Cytosol preparations from androgen-dependent tissues transferred ^{3}HDHT in a recoverable protein-bound form to nuclei of other androgen-dependent tissues but not to nuclei of androgen-independent tissues. No transfer of radioactive steroid from cytosol of these latter tissues to any nuclei was achieved. Labeled cytosol preparations from androgen-dependent tissues stimulated the RNA polymerase activity of nuclei from androgen-dependent tissues but not that of nuclei from androgen-independent tissues. Cytosol preparations from these latter tissues had no effect on RNA polymerase activity. Under suitable ionic conditions, human cytosol preparations containing DHT stimulated both alpha-amanitin-sensitive and -insensitive RNA polymerase activities of human prostatic nuclei. However, rat ventral prostatic DHT-cytosol protein complexes were equally as efficient in performing this function, suggesting the possible involvement of specific DHT-receptor complexes in this process. The authors suggest that receptor molecules from androgen-dependent tissues may not

be specific but may share properties which would facilitate research into the understanding and etiology of pathological conditions.

148. METABOLISM AND PROLIFERATIVE ACTIVITY OF TESTOSTERONE (MEETING ABSTRACT).

Alison MR, Taylor W, Wright NA, Appleton DR
Physiology-Medical Statistics, Univ. Newcastle upon Tyne, Newcastle upon Tyne, NE1 4LP, England
J Endocrinol; 167(2):8P-9P 1975

The in vivo metabolism of (7-alpha**-3 H)testosterone in the seminal vesicle and coagulating gland was studied in mice castrated 3 and 14 days before injection of the hormone. Two wk after castration, 85% of the target cells in the coagulating gland and 100% of those in the seminal vesicle were androgen-responsive. In intact controls, testosterone increased glandular weight but induced no proliferative activity. In the controls (0.5 hr after injection), 80% of the radioactivity per unit of DNA associated with nonpolar steroid corresponded to the dihydrotestosterone fraction. In the 3-day castrated mice, uptake was increased 4.7-fold in the seminal vesicle and 2-fold in the coagulating gland over precastration values. In the 14-day castrated mice, maximum values were much reduced from the 3-day mice, probably due to a reduction in cytoplasmic receptor protein associated with each nucleus.

149. A SPECIFIC 17B-ESTRADIOL RECEPTOR IN HUMAN BENIGN HYPERTROPHIC PROSTATE.

Bashirelahi N, O'Toole JH, Young JD
Dept. Biochemistry, Sch. Dentistry, Univ. Maryland, Baltimore, MD 21201
Biochem Med; 15(3):254-261 1976

A specific 17b-estradiol receptor was detected in the cytosol of human benign hypertrophic prostate. Scatchard analysis of the whole cytosol indicated that the receptor(s) had a dissociation constant of 1.73 x 10**-9 M and a binding site concentration of 12 x 10**-15 moles/mg of cytosomal protein. Specific nuclear uptake of ^{3}H-labeled estradiol from the cytosol was observed in the temperature range of 21-35 C but not at 0 or 45 C. The temperature dependence noted with human prostate nuclei and the specificity of uptake appear to be indicative of a specific 17b-estradiol-binding protein in the cytosol from human prostate. Evidence against sex hormone binding globulin involvement in the cellular binding of estradiol was obtained from data on protamine sulfate precipitation and data on the fractionation of the raw cytosol and serum. (15 refs)

150. THE EFFECTS OF ANDROGENS AND ANTIANDROGENS ON HORMONE-RESPONSIVE HUMAN BREAST CANCER IN LONG-TERM TISSUE CULTURE.

Lippman M, Bolan G, Huff K
Medicine Branch, National Cancer Institute, Bethesda, Maryland 20014
Cancer Res; 36(12):4610-4618 1976

We have examined five human breast cancer cell lines in continuous tissue culture for androgen responsiveness. One of these cell lines shows a 2- to 4-fold stimulation of thymidine incorporation into DNA, apparent as early as 10 hr following androgen addition to cells incubated in serum-free medium. This stimulation is accompanied by an acceleration in cell replication. Antiandrogens cyproterone acetate (6-chloro-17 alpha-acetate-1,2alpha-methylene-4,6-pregnadiene-3,20-dione) and R2956 (17 beta-hydroxy-2,2,17 alpha-trimethoxyestra-4,9,11-triene-1one)) inhibit both protein and DNA synthesis below control levels and block androgen-mediated stimulation. Prolonged incubation (greater than 72 hr) in antiandrogen is lethal. The MCF-7 cell line contains high-affinity receptors for androgenic steroids demonstrable by sucrose density gradients and competitive protein binding analysis. By cross-competition studies, androgen receptors are distinguishable from estrogen receptors also found in this cell line. Concentrations of steroid that saturate androgen receptor sites in vitro are about 1000 times lower than concentrations that maximally stimulate the cells. Changes in quantity and affinity of androgen binding to intact cells at 37 degrees as compared with usual binding techniques using cytosol preparation at 0 degrees do not explain this difference between dissociation of binding and effect. However, this difference can be explained by conversion of (^{3}H)-5alpha-dihydrotestosterone to 5alpha-androstanediol and more polar metabolites at 37 degrees. An examination of incubation media, cytoplasmic extracts, and crude nuclear pellets reveals probable conversion of (^{3}H)testosterone to (^{3}H)-5alpha-dehydrotestosterone. Our data provide compelling evidence that some human breast cancer, at least in vitro, may be androgen dependent. (Author Abstract)

151. THE METABOLISM AND BINDING OF TESTOSTERONE IN ANDROGEN-DEPENDENT AND AUTONOMOUS TRANSPLANTABLE MOUSE MAMMARY TUMORS.

Bruchovsky N, Meakin JW
Dept. Med., Univ. Alberta, Edmonton, Alberta, Canada
Cancer Res; 33(7):1689-1695 1973

Male mice bearing the SC-115 androgen-dependent mouse mammary carcinoma (Dm) and male and female mice bearing autonomous tumors derived from SC-115 (Am and Af, resp) were inj with testosterone-1,2-^{3}H (200 microCi, iv). Radioactivity incorporated into the tumors reached a peak within 15 min, then declined at approx the same rate in both types of tumors. Thirty min after inj, the radioactivity was similar in intact Dm, Am, and Af tumors, but the radioactivity in nuclei of Dm tumors was almost 4x that in nuclei of Am and Af tumors. Calculations of the recovery of radioactivity at each step of purification of the neutral metabolites of testosterone suggest that the amount of polar metabolites formed in each type of tumor cell is similar. The overall radioactivity recovered in the purified neutral fraction from nuclei was 2-4x the recovery from cytoplasm; it appears that the nuclei of tumor cells retained the ability to exclude polar metabolites. Testosterone represented 69.7-71.8% of the neutral steroids in the cytoplasm and 86.3-89.7% in the nuclei in Dm, Am, and Af tumors. Other metabolites identified were dihydrotestosterone, androstanedione, androstenedione, and androsterone. Radioactive androgens bound to steroid-binding protein were 4x higher in the cytosol of Dm cells than in Am cells, and there was a corresponding difference in the specific activities of the binding proteins. In Dm and Am nuclei, the amount of binding was almost equal when measured as a percentage of the total radioactivity in the nuclear fraction, but the specific radioactivity of the nuclear protein was approx. 4x higher in Dm nuclei (as expected from the greater incorporation of radioactivity demonstrated in the Dm nuclei). The steroid composition of the binding protein was similar to the steroid constituents in nuclei. These results are consistent with the view that the impaired incorporation of androgens into nuclei of autonomous cells is related to a reduced concn of cytoplasmic receptors. (51 refs)

152. HUMAN BREAST CANCER RESPONSIVE TO ANDROGEN IN LONG TERM TISSUE CULTURE.

Lippman ME, Bolan G, Huff K
Med. Branch, NCI, Bethesda, Md.
Nature (Lond); 258(5533):339-340 1975

Enhancement of precursor incorporation into macromolecules by androgens is reported in MCF-7, a human breast cancer cell line in tissue culture. 5-a-Dihydrotestosterone (10**-6 M) stimulated thymidine and

leucine incorporation approx. 4 and 2.5-fold, resp. The stimulation was less with testosterone (10**-6 M), and 5-b-dihydrotestosterone was ineffective. 17-b-hydroxy -2,2,17- a-trimethyl-estra -4,9,11-1 trien -3- one (10**-6 M) was inhibitory to thymidine incorporation. Net protein synthesis and cell division were also stimulated. Binding studies on cytoplasmic extracts using a dextran-coated-charcoal assay revealed high-affinity, androgen-binding molecules with affinity for both 5-a-dihydrotestosterone and testosterone (Kd = 7.5 x 10**-10 M, r = 0.997 for 5-a-dihydrotestosterone; corresponding values for testosterone were 1.5 x 10**-9 M and 0.958). It is suggested that the reduced form may be the active compound, and a 5-a-reductase activity was demonstrated. Cross competition studies strongly suggested separate receptor sites for estrogen and androgen in these cells. Some pts with metastatic breast cancer might benefit from adrenalectomy, thus removing weak androgen precursors which can be transformed into more potent androgens. Examination of human tumors for androgen receptors might lead to better therapeutic decisions for these pts. (18 refs)

153. STEROID RECEPTOR PROTEINS AND REGULATION OF GROWTH IN MAMMARY TUMORS.

Bruchovsky N, Van Doorn E
No affiliation given
Recent Results Cancer Res; 57:121-142 1976

The regulation of mammary tissue growth by steroid hormones and relevant experimental results obtained with two animal model systems, the rat ventral prostate gland and the androgen-dependent Shionogi mouse mammary tumor, are discussed. In some hormone-resistant tumors, the resistance may result from lack of entry of the hormone into the nucleus, which seems to correlate with a lack of cytoplasmic steroid receptor protein. In others, the interaction of hormone with chromatin is probably abnormal. This interaction may control three growth responses: (1) initiation of DNA synthesis and cell proliferation; (2) the switching-off of DNA synthesis when a tissue reaches normal size (negative feedback); and (3) autophagia. The expression of these responses may be partly or totally deficient in tumors. (28 Refs)

E. The Progesterone Receptor in Normal and Neoplastic Tissues

154. CHARACTERIZATION AND ASSAY OF PROGESTERONE RECEPTOR IN HUMAN MAMMARY CARCINOMA.

Pichon MF, Milgrom E
Groupe de Recherches sur la Biochimie Endocrinienne et la Reproduction (INSERM U. 135), Faculte de Medecine, Paris-Sud, 94270 Bicetre, France
Cancer Res; 37(2):464-471 1977

(³H)Pregn-4-ene-3,20-dione ((³H)progesterone)-receptor complexes from human mammary carcinoma were found to be stabilized in the presence of glycerol. The dissociation rate constant was lowered and the equilibrium dissociation constant was decreased (K D = 3 nM in the absence of glycerol and 1.1 nM in the presence of 30% glycerol), whereas no clear-cut effect on the association rate was observed and no change occurred in the concentration of binding sites. Cortisol was found to compete with (³H)progesterone only at concentrations higher than 1 uM. This made it possible to distinguish (³H)progesterone binding to the receptor from binding to corticosteroid-binding globulin. Synthetic progestins (6-chloro-17-acetoxypregna-4,6-diene-3,20-dione (chlormadinone acetate), 17alpha-ethinyl, 17-hydroxyestr-4-en-3-one (norethisterone), and 17,21-dimethyl-19-norpregna-4,9-diene-3,20-dione (R5020)) were found to have a high affinity for the receptor, whereas 5alpha-pregnane-3,20-dione had an affinity about one-half that of progesterone itself. 5beta-Pregnane-3,20-dione, 17alpha-hydroxypregn-4-ene-3,20-dione (estradiol), 11beta,21-dihydroxypregn-4-ene-3,20-dione (corticosterone), estra-1,3,5(10)-triene-3,17beta-diol, and 17beta-hydroxyandrost-4-en-3-one (testosterone) were weak inhibitors of (³H)progesterone binding. Sedimentation on glycerol gradients showed different patterns in different tumors; ie, (³H)progesterone specific binding having the characteristics of receptor was found either in the 8 S region, in the 4.5 S region, or in both. Activated progesterone-receptor complex from human mammary carcinoma cytosol was shown to bind to human DNA. An assay of the receptor based on these binding properties is described. This assay measures the total concentration of cytosol receptor since it makes possible the exchange of endogenous hormone for excess added (³H)progesterone. Of 55 biopsies examined by this method, 35 (64%) had a concentration of progesterone receptor-binding sites higher than 10 fmoles/mg protein. There was a positive correlation between the amounts of estrogen and progesterone receptors. (Author Abstract)

155. THE DETERMINATION OF PROGESTERONE RECEPTORS IN BREAST CANCER AND THEIR RELATIONSHIP TO ESTROGEN RECEPTORS.

Liskowski L, Rose DP, Dondlinger T, Olenick JS
Div. Clinical Oncology, Wisconsin Clinical Cancer Center, Univ. Hosp., Madison, WI 53706
Clin Chim Acta; 71(2):309-318 1976

Specific progesterone receptors (PR) in breast cancer tissue from 74 patients (aged 29-92 yr) were assayed using a quantitative procedure for observing the translocation of ³H-labeled progesterone into the tissue nuclear fraction. The assay utilized optimal binding conditions while avoiding interference from corticosteroid-binding globulin and glucocorticoid receptors which also have affinity for progesterone. The PR levels ranged from 0-25.3 femto (f)mol/mg protein, and receptors were detectable in 85% of the 74 breast cancer cases. Concentrations above 3.0 fmol/mg protein were detected in 73% of the cases. There was no relationship between PR and either the patient's age or site of tumor. There was no linear correlation between the levels of PR and estrogen receptors (ER). However, when the tumors were classified into those with or without detectable ER (above 1 fmol/mg protein), negative samples frequently showed low PR values; 75% were between 0 and 3.0 fmol/mg protein. When the ER results were plotted according to whether or not the tumors contained a PR level above 3.0 fmol/mg protein, 40% of the samples with PR concentrations above 3.0 fmol/mg protein had ER concentrations below 10 fmol/mg protein. (19 refs)

156. A BIOCHEMICAL BASIS FOR SELECTING ENDOCRINE THERAPY IN HUMAN BREAST CANCER. (PP. 177-186)

McGuire WL, Horwitz KB, De La Garza M
Breast Cancer, Trends in Research and Treatment. A Monograph of the European Organization for Research on Treatment of Cancer. New York, Raven Press, 1976.

In estrogen target tissues and hormone-dependent tumors, the steroid enters the cells and binds to a cytoplasmic protein called the estrogen receptor (ER). The steroid-receptor complex then migrates to the nuclei where it initiates the biochemical events characteristic of estrogen stimulation. Since ER is absent in tissues not responsive to estrogen, studies have been designed to show whether ER in human breast cancer tissue might be used to identify those patients likely to respond to endocrine therapy. Two properties of the ER (high-affinity binding of ³H-estradiol and sedimentation of receptor

primarily at 8S in low-salt sucrose gradients) have been used to quantitate ER in cancer specimens (700). Positive ER values are found in 70% of primary specimens and 58% of metastatic specimens. Other assays have also been performed and correlated with clinical response; data on 436 treatment trials in 380 patients indicate that if a patient's tumor does not contain ER there is virtually no chance of tumor regression after endocrine therapy. When tumors have ER, 55-60% of patients respond to such therapy as surgical ablation (castration, adrenalectomy, hypophysectomy), additive therapy (estrogens, androgens, glucocorticoids), and miscellaneous therapy (antiestrogens, aminoglutethimide). This evidence, coupled with menopausal status, disease-free interval, site of dominant lesion, and response to previous hormonal therapy, should allow selection of endocrine therapy. It may also be possible to identify the 40% of the ER-positive tumors that are endocrine resistant by means of a progesterone receptor (PgR) marker, thus sparing patients unrewarding therapies. It is noted that postmenopausal women have a higher ER level than premenopausal women. No survival data changes are expected with the use of ER measurements. In a discussion of endocrine aspects, correlations between treatment and other receptors (androgen, estradiol) are noted, but there is no correlation with number of binding sites and response between the amount of PgR and response to endocrine treatment. (18 refs)

157. STEROID-BINDING PROTEINS OF THE MAMMARY GLAND AND THEIR CLINICAL SIGNIFICANCE IN BREAST CANCER.

Wittliff JL, Mehta RG, Boyd PA, Goral JE
Dept. Biochemistry, Univ. Rochester Medical Center, Rochester, NY 14642

J Toxicol Environ Health; Suppl. 1: 231-256; 1976

Differentiation and development of the mammary gland are influenced by estrogens, glucocorticoids, and progesterone. Specific binding sites for each of these classes of steroid hormones were characterized in vivo and in cell-free preparations of lactating mammary gland of the rat. These properties were compared with those of steroid-binding proteins in the R3230AC and dimethylbenz(a)anthracene-induced mammary tumors of the rat and of human breast carcinomas. The binding components that were protein in nature were more concentrated in tissue from lactating rats than from virgin or pregnant rats. The steroid-binding capacities of mammary tumors were highly variable. Using a dextran-coated charcoal procedure, the rate constants of ^{3}H-ligand association with specific binding sites were temperature-dependent. Ligand specificity indicated that ^{3}H-estradiol-17b (^{3}H-E) binding was inhibited only by estrogens, whereas ^{3}H-triamcinolone acetonide (^{3}H-TA) was blocked by glucocorticoids and progesterone. ^{3}H-progesterone (^{3}H-P) binding was inhibited by progestins and certain glucocorticoids. Incubation of minced mammary gland with ^{3}H-steroid in the presence and absence of unlabeled competitor demonstrated specific binding sites in cytoplasm that were translocated to nuclei in a temperature-dependent fashion. Using a steroid-exchange procedure, both DEX and TA inhibited ^{3}H-P binding to exchangeable sites that were previously loaded with unlabeled progesterone. These data suggest that certain glucocorticoid and progesterone binding sites were translocated simultaneously to nuclei. Administration in vivo of ^{3}H-E or ^{3}H-TA indicated binding in cytoplasm and subsequent translocation to nuclei. It is concluded that analysis of estrogen receptors in a breast tumor biopsy can be a useful index for the selection of endocrine therapy for patients with advanced breast carcinoma. (34 refs)

158. PROGESTERONE AND PROGESTERONE RECEPTORS IN EXPERIMENTAL BREAST CANCER.

Horwitz KB, McGuire WL
Dept of Medicine, Univ of Texas Health Science Center, San Antonio, TX 78284

Cancer Res; 37(6):1733-1738 1977

The synthetic progestin, R5020, has been used to demonstrate a progesterone receptor (PGR) in 7,12-dimethylbenz(alpha)anthracene-induced rat mammary tumors which binds hormone with high affinity (Kd approx 1 nM) and migrates at 7S and 4S on sucrose density gradients. Rats bearing growing tumors were ovariectomized-adrenalectomized at proestrus, tumors were biopsied 24 hr later, and estrogen receptor and PGR were determined by a single, saturating-dose dextran-coated charcoal assay. PGR values averaged 247 plus or minus 27 fmoles/mg cytosol protein. When rats received injections of progesterone plus estradiol, biopsied tumors resumed growth and PGR levels were maintained. PGR was also unchanged in tumors growing on estradiol alone. Tumors regressed after withdrawal of progesterone + estradiol or of estradiol alone, and PGR uniformly fell to basal levels (less than 50 fmoles/mg cytosol protein). Estrogen treatment of regressed tumors restored both tumor growth and PGR. In sum, castration with adrenalectomy induced rapid tumor regression and loss of PGR. Estradiol administration prevented tumor regression and PGR loss or restored PGR in regressed tumors. Progesterone alone failed to sustain tumor growth despite the initial presence of PGR. (24 Refs) (Author Abstract)

159. PROPERTIES OF PROGESTERONE-BINDING PROTEINS IN MAMMARY TISSUES.

Goral JE, Turnell RW, Wittliff JL
Department of Biochemistry and University of Rochester Cancer Center, University of Rochester Medical Center, Rochester, New York 14642

Proc Am Assoc Cancer Res; 16:154 1975

Kinetic and molecular properties of ^{3}H-progesterone (P)-binding proteins were determined in cell-free preparations of lactating mammary glands, R3230AC mammary adenocarcinoma and dimethylbenz(a)anthracene (DMBA)-induced mammary tumors of rats. Using 10mM Tris-HCl buffer, pH 7.4, containing 1.5 mM EDTA and 10 mM monothioglycerol as a homogenizing medium and a dextran-coated charcoal adsorption procedure to measure binding, ^{3}H-P associated maximally with specific sites in cytosols from each tissue within 2 hr. Binding was of limited capacity (150-650 fmol/mg cytosol protein) and high affinity (approximately equal to 10**-8M). Unlabeled triamcinolone acetonide (TA) was competitive for ^{3}H-P binding sites, suggesting that the component was not transcortin. Hydrocortisone and corticosterone were competitive inhibitors although aldosterone, dihydrotestosterone and estradiol-17beta were inhibitory only at 10^3-fold excess. ^{3}H-P-binding components sedimented at 4-5S on sucrose gradients of either low or high ionic strength. Titration of the same cytosol with either ^{3}H-P or ^{3}H-TA gave similar numbers of binding sites. These data and those indicating that progesterone and glucocorticoids compete for the same binding sites suggest that these hormones may influence mammary gland differentiation and development by a common mechanism. (Author Abstract)

160. IDENTIFICATION OF PROGESTERONE RECEPTORS IN HUMAN MALIGNANT BREAST TUMOURS AND IN HUMAN UTERINE TISSUE (MEETING ABSTRACT).

Poortman J, Vroegindewey-Jie D, Meddens M, Thijssen JH
Dept. Endocrinology, Univ. Hosp., Catharijnesingel 101, Utrecht, Netherlands
J Endocrinol; 72(1):53P 1977

Preliminary results of previous studies indicated that the specific progesterone receptor (PgR) may be an effective marker for the response to endocrine treatment of malignant breast tumors. Studies were thus performed to develop a routine assay for the simultaneous estimation of the oxygen enhancement ratio (OER) and PgR in human mammary tumors. Normal human uterine tissue was used as a control. Tritium labeled progesterone and R-5020 were used for the specific receptors in human mammary tumors. High speed cytosol was incubated with various concentrations of the labeled compounds and in the presence of a 100-fold excess of the non-radioactive compounds. The free and bound steroids were separated by either gel-electrophoresis or by sucrose gradient centrifugation by dextran-charcoal. R-5020 was helpful in avoiding specific binding due to contaminating plasma proteins. It was not absolutely specific for PgR. Progesterone receptors sedimented at about 4 S in a 0.4 M KCl sucrose gradient with R-5020. The 4 S progesterone was less inhibitory for R-5020 binding than R-5020 itself. There were more PgR in myometrial tissue than in most uteri; they were present in OER positive and negative breast tumors. These results indicate that R-5020 cannot yet be used for a simple charcoal assay for the specific determination with progesterone receptors. (1 ref)

161. PROGESTERONE BINDING IN HUMAN ENDOMETRIAL CARCINOMAS.

Young PC, Ehrlich CE, Cleary RE
Medical Research Faculty, Room 103, Indiana University Medical Center,Indianapolis, Indiana 46202
Am J Obstet Gynecol; 125(3):353-360 1976

By means of dextran-coated charcoal assay, the capacity of various endometrial cytosol preparations for specific binding of ^{3}H-progesterone was determined. With the use of an arbitrary value of 50 femtomoles of bound ^{3}H-progesterone per milligram of cytosol protein as the breaking point between high and low binding capacities, 19 out of 20 normal endometria had high progesterone-binding capacities. Two out of 11 Grade I, 3 out of 8 Grade II, and 2 out of 4 Grade III endometrial carcinomas showed low binding capacities. All 4 endometrial polyps, 7 out of 9 hyperplastic endometria, and 0 out of 7 nonendometrial gynecologic tumors had high binding capacities. These data suggest a progressive loss of specific progesterone-binding activity from normal endometria to hyperplastic endometria, and from the well-differentiated to the anaplastic forms of endometrial adenocarcinoma. There seemed to be an inverse relationship between age and concentration of progesterone receptors in endometrial adenocarcinomas. All irradiated tumors studied had low progesterone-binding capacity. (Author Abstract)

162. THE MODE OF ACTION OF PROGESTAGENS ON ENDOMETRIAL CARCINOMA. (PP. 85-94)

Reel JR
Steroid Hormone Action and Cancer. Menon KM, Reel JR, ed. New York, Plenum Press, Current Topics in Molecular Endocrinology, vol. 4, 1976.

The mode of action of progestagens on endometrial carcinoma at the cellular and molecular levels is reviewed. Short-term incubations and organ and cell culture systems both have been used to study the direct action of progestagens on human endometrium or endometrial carcinoma. Acute exposure of normal endometria to high concentrations of progesterone was cytotoxic and inhibited nucleic acid synthesis, while in endometrial carcinoma, progesterone produced a dose-dependent decrease of nucleic acid synthesis and cell survival. Inhibitory action on cell survival was potentiated by 17b-estradiol, especially in poorly differentiated carcinomas. In vivo estrogen-progestagen treatment (80 mg polyestradiol phosphate with or without 1,250 mg 17a-hydroxyprogesterone caproate given to cancer patients twice weekly, with or without 300 mg 19-nor-17a-hydroxyprogesterone caproate twice weekly for 3-7 wk) also inhibited RNA synthesis (in all patients) and DNA synthesis (in all but 2 patients). The relative responses of DNA and RNA synthesis in vitro and in vivo were highly correlated. Work has also been done with progestagen receptors, which might have prognostic value in determining which patients would respond to progestational therapy; these receptors have been found in normal and in hyperplastic and carcinomatous endometria. Data from studies of the uterus of humans, sheep, rats, mice, hamsters, and guinea pigs indicated that the progesterone receptor concentration of the uterus is under dual hormonal control: 17b-estradiol exerts a positive control (increases receptor concentration) and progesterone exerts a negative control (lowers receptor levels). In addition to being estrogen-inducible, human uterine progesterone receptors share many physicochemical properties in common with chick oviduct and other mammalian uterine receptors. The few data available on the presence of receptors in hyperplastic and carcinomatous endometria indicate variable or no binding capacity in these endometria: 4 of 10 carcinomas contained progesterone binding proteins, a fraction approximating the remission rate after progestational therapy. Though progestagen receptors are necessary, they may not be sufficient for hormone-induced remission, as demonstrated by a 55-60% response rate of breast tumors with estrogen receptors and by improved response to progestagens after priming with estrogens. (73 refs)

163. MULTIPLE BINDING SITES FOR PROGESTERONE IN THE HEN OVIDUCT NUCLEUS: EVIDENCE THAT ACIDIC PROTEINS REPRESENT THE ACCEPTORS. (PP. 153-186)

Spelsberg TC, Webster R, Pikler GM
Chromosomal Proteins and Their Role in the Regulation of Gene Expression. Stein GS Kleinsmith LJ, ed. New York, Academic Press Inc, 1975.

The identification and characterization of the acceptor for the progesterone receptor complex in oviduct nuclei of mature laying hens were investigated. The in vitro nuclear binding of the P-receptor to isolated nuclei from the oviducts of laying hens had the same properties and requirements as was reported earlier for the immature chick system. The differences in extent of binding to the nuclear material between a target tissue and other tissues were markedly dependent on the ionic conditions. The assay of the nuclear binding, using multiple levels of P-receptor, revealed the presence of more than one binding site in the oviduct nuclei. The hormone binding to each of the sites displayed a differential stability to increasing levels of ionic strength. Only the highest affinity binding site was capable of binding P-receptor under the higher ionic conditions. The multiple binding sites for the P-receptor were not found in the nuclei of a nontarget organ (spleen). Oviduct chromatin displayed the multiple binding sites as did whole nuclei. New techniques for assaying the binding of P-receptor to soluble nucleoproteins were developed. Using antibiotics to precipitate the DNA and nucleoproteins or the attaching of the latter to insoluble resins allowed the rapid assay of P-receptor binding with a minimal background. Using these

techniques, binding to dehistonized oviduct chromatin (containing acidic protein fractions AP3 and AP4) demonstrated that the highest affinity binding site was still present. When the acidic protein AP3 was removed from the DNA, this highest affinity binding site was lost. These results concerning the AP3 fraction corroborate earlier reports on progesterone binding in the chick oviduct. (24 refs)

164. NUCLEAR BINDING OF PROGESTERONE IN HEN OVIDUCT

Pikler GM, Webster RA, Spelsberg TC
Department of Molecular Medicine, Mayo Clinic and Mayo Foundation, Rochester, MN 55901, U.S.A.
Biochem J; 156(2):399-408 1976

Steroid hormones, including progesterone, are known to bind with high affinity (Kd approximately equal to 1 x 10**-10M) to receptor proteins once they enter target cells. This complex (the progesterone-receptor) then undergoes a temperature- and/or salt-dependent activation which allows it to migrate to the cell nucleus and to bind to the deoxyribonucleoproteins. The present studies demonstrate that binding the hormone-receptor complex in vitro to isolated nuclei from the oviducts of laying hens requires the same conditions as do other studies of binding in vitro reported previously, e.g. the hormone must be complexed to intact and activated receptor. The assay of the nuclear binding by using multiple concentrations of progesterone receptor reveals the presence of more than one class of binding site in the oviduct nuclei. The affinity of each of these classes of binding sites range from Kd approximately equal 1 x 10**-9M-1 x 10**-8M. Assays using free steroid (not complexed with receptor) show no binding to these sites. The binding to each of the classes of sites displays a differential stability to increasing ionic concentrations, suggesting primarily an ionic-type interaction for all classes. Only the highest-affinity class of binding site is capable of binding progesterone receptor under physiological-saline conditions. This class represents 6000-10000 sites per cell nucleus and resembles the sites detected in vivo (Spelsberg, 1976, Biochem. J. 156, 391-398) which cause maximal transcriptional response when saturated with the progesterone receptor. The multiple binding sites for the progesterone receptor either are not present or are found in limited numbers in the nuclei of non-target organs. Differences in extent of binding to the nuclear material between a target tissue (oviduct) and other tissues (spleen or erythrocyte) are markedly dependent on the ionic conditions and are probably due to binding to different classes of sites in the nuclei. (Author Abstract)

165. NUCLEAR BINDING OF PROGESTERONE IN HEN OVIDUCT.

Webster RA, Pikler GM, Spelsberg TC
Department of Molecular Medicine, Mayo Clinic, Rochester, MN 55901, U.S.A.
Biochem J; 156(2):409-418 1976

The multiple classes of binding sites for the progesterone receptor complex in hen oviduct nuclei were found to be of chromatin origin. The highest-affinity, and presumably most physiologically important class, is localized in oviduct chromatin and contains approx 6000-10000 sites per nucleus. None of these sites is detected in spleen chromatin. Two new techniques were used for assaying rapidly the binding of steroid-receptor complexes to soluble deoxyribonucleoproteins in vitro. The extent of high-affinity binding by the nucleo-acidic protein fraction from spleen chromatin is as great as that by the nucleo-acidic protein from oviduct chromatin. Consequently the tissue-specific nuclear binding of the progesterone receptor is found not to be a consequence of the absence of the nuclear binding sites (acceptors) from chromatin of non-target tissue (spleen), but rather a result of complete masking of these sites. In the target-tissue (oviduct) chromatin, approx 70% of the high-affinity acceptor sites are also masked. Acidic proteins, and not histones, appear to be responsible for the masking of these acceptor sites. In addition, acidic proteins represent (or at least are an essential part of) these high-affinity sites in the oviduct nucleus. Pure DNA displays a few high-and many low-affinity binding sites. In support of previous work with immature chicks, the acidic protein fraction of the nucleo-acidic protein of hen oviduct appears to contain this high-affinity class of binding sites. Our results thus support the hypothesis that protein complexed with DNA, and not DNA alone, represents the high-affinity binding sites for the steroid-receptor complexes in nuclear chromatin. The lower-affinity classes of binding sites may represent DNA and/or other nuclear components. (Author Abstract)

166. DMBA AND PROGESTERONE BINDING IN BREAST AND UTERUS (MEETING ABSTRACT).

Barlow J, Funder JW
Medical Res. Centre, Prince Henry's Hosp., Melbourne, 3004, Australia
Clin Exp Pharmacol Physiol; 3(3):268-269 1976

Both the carcinogenic effect of 7,12-dimethylbenz (a)anthracene (DMBA) and the dependence of the resulting tumors upon hormone status have been described by various groups. Previously it has been demonstrated that DMBA potentiates the binding of tritiated progesterone (³ HP) in vitro in both mammary gland parenchymal cytosol and uterine cytosol. Several aspects of this potentiating effect have been subsequently studied in greater detail. Cytosols were prepared from uterine and breast tissue of adrenalectomized, ovariectomized Sprague-Dawley rats. In both tissues the specificity of the potentiation of ³ HP binding by DMBA was examined by competition experiments using unlabelled cortisol, aldosterone, dihydrotestosterone and estradiol. Unlabelled progesterone displaced ³ HP from DMBA-potentiated binding sites in both tissues; the other steroids were orders of magnitude less effective, with DHT being most, and estradiol least effective as competitors. DMBA potentiation of ³ HP binding was also examined in animals of different ages. Between weaning and sexual maturity the levels of binding in the absence of DMBA remain low and constant; potentiation is greatest at weaning and thereafter gradually declines. Estrogen priming (0-4 ug estrogen benzoate on days -2 and -1, sc in oil) results in an increase in the level of ³ HP binding to levels similar to those seen after DMBA potentiation. The phenomenon of DMBA potentiation in breast and uterine cytosol has also been demonstrated in Wistar rats and guinea pigs. These observations lend support to the hypothesis that DMBA is unmasking specific progesterone binding proteins; and that the carcinogenic effect of the compound may be mediated via an effect on progesterone binding.

167. CHARACTERISTICS OF PROGESTERONE-BINDING COMPONENTS IN NEOPLASTIC MAMMARY TISSUES OF THE RAT.

Goral JE, Wittliff JL
University of Rochester Cancer Center, Rochester, New York 14642
Cancer Res; 36:1886-1893 1976

Characteristics of (³H)progesterone-binding components were studied in cell-free preparations of two hormonally responsive tumors: the R3230AC mammary adenocarcinoma and 9,10-dimethyl-1,2-benzanthracene-induced mammary tumor of the rat. Progesterone-binding macromolecules from cytosols of both mammary neoplasms exhibited sedimentation coefficients of 3.5 to 4.0 S on sucrose gradients of either low or high ionic strength. From Scatchard analyses of titration data,

apparent dissociation constants of 4 to 6 x 10**-8 M were determined for ligand-binding protein complexes from either tumor. Specific progesterone-binding capacities varied considerably, ranging from 150 to 650 fmoles/mg of cytosol protein. Optimal binding of (^{3}H)progesterone was reached by 2 to 3 hr at 3 degrees, pH 7.4, and then decreased rapidly. Specificity studies indicated that cortisol, corticosterone, and triamcinolone acetonide competed effectively for (^{3}H)progesterone-binding. This suggested that (^{3}H)progesterone was bound largely to a macromolecule distinct from transcortin, which does not bind glucocorticoids containing 9alpha-fluoro groups. Aldosterone, as well as several androgens and estrogens, were weak competitors of binding except at high concentrations. The nature of the inhibition of progesterone-binding sites by triamcinolone acetonide and corticosterone was competitive. Concurrent titrations of (^{3}H)progesterone and (^{3}H)triamcinolone acetonide-binding sites demonstrated that their binding capacities were similar considering the relative stabilities of the complexes. These results, which indicated that progesterone and glucocorticoids compete for the same binding site, suggest that these hormones may influence mammary gland differentiation and development by a common mechanism. (Author Abstract)

168. FRACTIONATION OF DIVERSE STEROID-BINDING PROTEINS: BASIC AND CLINICAL APPLICATIONS. (PP. 51-67)

Sherman MR, Miller LK

Steroid Hormone Action and Cancer. Menon KM, Reel JR, ed. New York, Plenum Press, Current Topics in Molecular Endocrinology, vol. 4, 1976.

A number of techniques that can be used both to fractionate mixtures of steroid-binding proteins in amphibian, avian and mammalian target tissues and to evaluate their molecular wt, Stokes radii, axial ratios and relative net charges, are discussed. Analyses of competitive steroid binding by techniques that discriminate not only between bound and free steroid but among several steroid-binding proteins are exemplified by gel filtration and electrophoretic patterns for human best tumor cytosol and serum. The advantages of fractionating steroid receptors and other proteins by electrophoresis in gels of large but varied pore size are shown. Studies of the multiple forms of the chick oviduct progesterone receptor serve to illustrate some useful combinations of techniques: electrophoresis following gel filtration or elution from DEAE filters and gel filtration following protamine precipitation or ion exchange filtration. Although the first step in each sequence was performed on unfractionated cytosol, the results permitted a detailed comparison of the molecular parameters of the major subcomponents of the receptor, and the smaller steroid-binding unit. Thus, form II has the largest Stokes radius, highest axial ratio and the highest net negative charge in the pH range of 7.4 to 10.2. A lower net negative in gels and its greater ease of extraction from DEAE-cellulose or protamine precipitates. Recently, another combination of techniques (data not presented) permitted the demonstration that form II, but not form III, is converted to form V on exposure to CACl2 . (16 refs)

169. STEROID-RECEPTOR QUANTITATION AND CHARACTERIZATION BY ELECTROPHORESIS IN HIGHLY CROSS-LINKED POLYACRYLAMIDE GELS.

Miller LK, Diaz SC, Sherman MR

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Biochemistry (Washington); 14(20):4433-4443 1975

Conditions for discontinuous polyacrylamide gel electrophoresis have been defined in which progesterone receptors of chick oviduct cytosol and a variety of steroid-binding proteins from other sources are stable and amenable to quantitative analysis. The essential modifications from standard procedures include the use of: separation gels in which the cross-linking agent/acrylamide monomer = 15:85.; of glycerol (10%) in all phases of the Tris-glycine-HCl buffer system (pH 10.2) in the separation phase during electrophoresis at 0 C; and of a layer of a charged reducing agent, thioglycolate, beneath the sample layer. Electrophoresis of untreated oviduct cytosol labeled with H3-progesterone competing steroids revealed a heterodisperse slow peak and a sharp fast peak. Both peaks displayed steroid-binding specificity and saturability that are characteristic of intracellular receptors. Recovery of steroid from both the slow and fast components increased linearly with sample load up to 50 microliters of cytosol (1.2 mg of protein)/gel (6 mm diameter). The specific progesterone binding detected by this technique was comparable to that detected by charcoal-dextran treatment or ion exchange filtration. Relative electrophoretic mobilities of globular protein standards and steroid-protein complexes in cytosol and chick serum were measured in separation gels with total gel concentrations systematically varied from 5% to 15%. The value of apparent molecular wt obtained for the cytosol fast component was independent of the length of the separation gel, the presence of a stacking gel or prior exposure of the cytosol to KCl. It was higher than expected from the sedimentation coefficient of 4.2S in the same pH 10.2 buffer. Electrophoresis in 170-mm separation gels without stacking gels revealed that KCl extracts of protamine-precipitated cytosol contain a different receptor form, of lower net negative charge than the cytosol fast form. The results demonstrate the utility of electrophoresis in highly cross-linked gels of several concentrations to discriminate between various receptor forms and steroid-binding components of serum.

F. Mechanism of Action of the Glucocorticoid Receptor in Normal and Neoplastic Cells and Tissues

170. THE GLUCOCORTICOID RECEPTOR. (PP. 317-390)

Cake MH, Litwack G

Biochemical Actions of Hormones. Litwack G, ed. New York, Academic Press, vol. III, 1975.

Recent advances in the identification of the glucocorticoid receptor in the presence of other steroid binding proteins are reviewed, with emphasis on its properties and actions within the target organ cell or hormone-responsive cell in culture. The present stage of research concentrates on the understanding of events following glucocorticoid entry into the target cell and the biochemical phenomena released by binding to the intracellular receptor which ultimately must explain the action of the hormone in the organism. The specific topics covered include: hormone action in liver, thymus, and other responsive or sensitive cells; intracellular actions of glucocorticoids, including entry of steroid into target cell, formation of hormone receptor complex, identification of the glucocorticoid receptor, properties of the glucocorticoid receptor, activation of the glucocorticoid receptor complex, translocation and binding of the glucocorticoid receptor complex in the nucleus, intranuclear events, and recycling of the glucocorticoid receptor; analysis of structural aspects of the receptor binding site; methods of measuring the glucocorticoid receptor; tissue distribution of the glucocorticoid receptor; developmental changes in glucocorticoid receptor activity; and systems with altered receptor states. (174 refs)

171. BINDING OF GLUCOCORTICOID RECEPTORS TO DNA.

Rousseau GG, Higgins SJ, Baxter JD, Gelfand D, Tomkins GM

Departments of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143

J Biol Chem; 250(15):6015-6021 1975

DNA has been implicated as the nuclear acceptor for receptor-glucocorticoid complexes. The present study concerns the interaction of these complexes, isolated from cultured rat hepatoma cells, with purified DNA. This association is rapid, reaching a maximum within a few minutes at 0 degrees, whereas dissociation requires several hours. DNA binds neither free glucocorticoids nor those complexed with transcortin or cytosol proteins different from the receptor. Receptors which are not complexed by steroid have little or no affinity for DNA. "Activation," necessary for the binding of receptor-steroid complexes to isolated nuclei, also enhances DNA binding. The capacity of DNA for binding receptor-steroid complexes is large; saturation was not observed at the complex concentrations studied, using either crude or partially purified receptor preparations. The association of complexes with DNA is inhibited by divalent cations, at increasing ionic strengths, and by mercurial reagents. Complexes bind equally well to bacterial, bacteriophage, or rat DNA; however, there was either no or substantially reduced binding by bacterial 23 S rRNA. The binding of complexes to native DNA is roughly 3-fold greater than to denatured DNA. These characteristics are consistent with the possibility that DNA is the nuclear acceptor for receptor-glucocorticoid complexes; however, the actual composition of the acceptor sites remains unknown. (Author Abstract)

172. INTERACTIONS OF GLUCOCORTICOID RECEPTOR STEROID COMPLEXES WITH ACCEPTOR SITES.

Simons SS, Martinez HM, Garcea RL, Baxter JD, Tomkins GM

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J Biol Chem; 251(2):334-343 1976

The binding of the "activated" receptor-glucocorticoid complexes of cultured rat hepatoma cells to nuclei, chromatin, and DNA has been studied under cell-free conditions. A critical factor in determining the shape of the binding curve is shown to be an inhibitory material which is present in crude cytosol and which can be removed without destroying the receptor-steroid complex. These and other results argue that the apparent saturation observed in earlier experiments may have been due to the inhibitors. Thus, the actual number of acceptor sites in hepatoma tissue culture cell nuclei is much larger than previously estimated and their affinity for the complex is lower. Nuclear binding experiments indicated that the inhibitory material appear to be macromolecular; but their effects cannot be mimicked by albumin or hemoglobin. The acceptor capacity at low ionic strength for binding receptor-glucocorticoid complexes increases when proceeding from nuclei to DNA. An analysis of the kinetics of association and dissociation and of the relative binding behavior of nuclei and DNA argues that the affinity of complex for nuclei is much greater than for DNA. DNA associated histones reduce the amount of complex that binds to DNA. These and perhaps other chromosomal proteins may be responsible for the ordering of acceptor capacity. Evidence is presented that the difference in affinities of nuclear and DNA acceptors could also be due to chromosomal proteins. In nuclei these proteins may thus both reduce the amount of complex binding by rendering regions of DNA less accessible and increase the binding affinity of some, or all, of those DNA binding sites which remain exposed.

173. THE EFFECTS OF GLUCOCORTICOIDS AND PROGESTERONE ON HORMONE-RESPONSIVE HUMAN BREAST CANCER IN LONG-TERM TISSUE CULTURE.

Lippman M, Bolan G, Huff K

Medicine Branch, National Cancer Institute, Bethesda, Maryland 20014

Cancer Res; 36(12):4602-4609 1976

Glucocorticoids, at physiological concentration, inhibit cell division and thymidine incorporation in three lines of human breast cancer maintained in long-term tissue culture. At steroid concentrations sufficient to inhibit thymidine incorporation 50%, little or no effect is seen on protein synthesis 48 hr after hormone addition. All three of these lines are shown to have glucocorticoid receptors demonstrable by competitive protein binding assays. Receptors are extensively characterized in one line by sucrose density gradient analysis and binding specificity studies. Good correlation between receptor-binding specificity and biological activity is found except for progesterone, which binds to glucocorticoid receptor but is noninhibitory. Cross-competition and quantification studies demonstrate a separate receptor for progesterone. This receptor has limited binding specificities restricted largely to progestational agents, whereas the glucocorticoid receptor bound both glucocorticoids and progesterone. Two other human breast cancer lines neither contain glucocorticoid receptor nor are inhibited by glucocorticoids. It is concluded that in some cases glucocorticoids can directly limit growth in human breast cancer in vitro without requiring alterations in other trophic hormones. (Author Abstract)

174. RESTORATION OF WILD-TYPE RESPONSE TO STEROIDS IN L CELLS LACKING STEROID RECEPTOR BY HYBRIDIZATION WITH NORMAL L CELLS.

Thompson EB, Venetianer A

Natl. Cancer Inst., Bethesda, MD 20014

J Cell Biol; 67:431a 1975

Wild-type L cells displayed inhibition of thymidine incorporation and of cloning efficiency following exposure to glucocorticoid hormones. These responses required that the cells possess a cytoplasmic receptor protein for such steroids. Steroid-resistant, receptorless (R-) L cells could be isolated by cloning wild-type cells in high concentrations of dexamethasone. One such clone, SLB82-17, has been used in the genetic analysis of steroid responsiveness. In published work, receptor-containing rat hepatoma (HTC) cells, possessed of the steroid-inducible enzyme tyrosine aminotransferase, but not showing the inhibitory responses of L cells, were hybridized with the SLB82-17 R- cells. There was no restoration of the L cell-specific responses. It is possible that the HTC cell introduced a repressor of the L cell responses, or the HTC cell receptor differed from and could not substitute for the L cell receptor, or the SLB82-17 cells, besides being R-, were unable to respond even when supplied with functional receptor. The present experiments were done to test the latter possibility. SLB82-17 R- cells were fused by standard methods with LA9 R+ cells, a series of hybrid clones isolated and tested for steroid responsiveness and receptor content. By conventional in vitro competitive assay for receptor content, several of the hybrids showed little or none. Yet in vivo tests showed that they took up steroid and transferred it to the nucleus normally. Extracts of these clones, moreover, were found to contain an inhibitor of receptor function, apparently released during homogenization. Cloning

efficiency and thymidine incorporation indicated that all the hybrids had the normal L cell responses to steroids. Thus the R line does not contain a dominant repressor of steroid responsiveness, and receptor of L cell origin can function in the presence of the SLB82-17 genome.

175. STEROID HORMONE RECEPTORS: A SURVEY.

Mainwaring WI

Imperial Cancer Res. Fund. Lincoln's Inn Fields, London, England

Vitam Horm; 33:223-245 1975

A simplified model for the action of steroid hormones, including uptake, metabolism, binding to a high-affinity receptor, activation, and molecular action, is presented and discussed. Antagonists for many steroids are now available that counter the formation of the receptor complex and negate virtually all of the hormonal response. Differences are cited in the receptor contents of experimental tumors that may exist in glucocorticoid-sensitive and -insensitive lines. Credence in receptor systems is drawn from the striking correlation between steroid-binding receptors and biological activity. Evidence for and against an entry mechanism for steroids is discussed. Steroid antagonists are of interest as potential chemotherapeutic agents and as research tools. Regarding steroid metabolism in the model system, only for the androgens can a convincing case be made for mandatory involvement. At this point, the purification of steroid-receptor complexes is urgent in further elucidation of the mechanisms of action of steroid hormones. Conventional approaches to this problem and affinity chromatography are discussed. Further effort is also required to provide a satisfactory explanation for tissue specificity of hormonal responses.

176. STUDIES ON THE HEPATIC GLUCOCORTICOID RECEPTOR AND ON THE HORMONAL MODULATION OF SPECIFIC MRNA LEVELS DURING ENZYME INDUCTION.

Feigelson P, Beato M, Colman P, Kalimi M, Killewich LA, Schutz G

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Recent Prog Horm Res; 31:213-242 1975

Recent evidence is reviewed which provides certain insights into the nature of the events by which the glucocorticoid receptors may mediate hormonally controlled processes. The presence of protein has been demonstrated in hepatic cytosol which binds glucocorticoid hormones, both natural and synthetic, with high affinity and which has appropriate stereospecificity. The steroid-receptor complex undergoes a temperature-dependent activation enabling it to bind to DNA and nuclei. This activation process is accelerated by physiologic levels of calcium. The finding that administering glucocorticoids to animals causes translocation of receptor molecules from the cytoplasm into the nucleus supports the important role played by the receptor protein. The loss of receptor molecules from the cytoplasm is proportional to the degree of physiologic response in the liver, that is, the degree of enzyme induction. Neither the glucocorticoid receptor nor the steroid alone binds to nuclei or DNA. However, the activated glucocorticoid receptor complex will. Additional facts indicate that treatment results in a selectively augmented rate of synthesis of the inducible enzyme, tryptophan oxygenase, mediated by a rise in the steady-state level of the functional mRNA coding for this enzyme protein. These findings are compatible with the theory that the steroid may act by accelerating the transcription of specific genes which code for the inducible proteins. It is at present possible to conclude rather convincingly that glucocorticoid hormones, at least in the hormonal induction of hepatic tryptophan oxygenase, augment the rate of synthesis of this inducible enzyme by elevating the tissue level of its functionally active mRNA.

177. DISTRIBUTION OF THYROID AND GLUCOCORTICOID HORMONE RECEPTORS IN TRANSCRIPTIONALLY ACTIVE AND INACTIVE CHROMATIN.

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Biochem Biophys Res Commun; 68(4):1045-1051 1976

The distribution of glucocorticoid and thyroid hormone receptors in partially purified transcriptionally active and inactive chromatin fractions obtained after shearing chromatin from cultured pituitary (GC) cells with deoxyribonuclease II (DNase) was investigated. After DNase II shear and centrifugation of GC cell chromatin, about 10% of the DNA was released onto the supernatant (S1). This fraction is greatly enriched (20-fold) in nascent RNA chains. The protein/DNA ratio of both S1 and P1 (unsheared chromatin) fractions is about 1, similar to that of unfractionated chromatin. Chromatin containing bound I125-triiodothyronine (T3) was fractionated and showed that the major proportion of specifically bound T3 is found in P1 or transcriptionally inactive chromatin. Only 10-20% of the radioactivity was released into the supernatant by the nuclease action. The specific activity of receptors was 50% higher in fraction S1 ; however, the actual specific activity of S1 and P1 is probably the same, since some bound T3 may dissociate during the brief 24 C incubation with the DNase. The distribution of 90% glucocorticoid receptors are located in the genetically inactive chromatin fractions. The specific activity of dexamethasone in fraction S1 varied from 1 to 3. The results suggest that by shearing chromatin with DNase II, two chromatin fractions are obtained which have marked differences in their transcriptional activity. (21 refs)

178. HORMONAL REGULATION OF GENE EXPRESSION. (PP. 3-7)

Tomkins GM

Differentiation and Control of Malignancy of Tumor Cells. Proceedings of the International Symposium of the Princess Takamatsu Cancer Research Fund, 4th. Baltimore, University Park Press; 1975.

Experimental work on the regulation of gene expression by the adrenal glucocorticoids is reviewed. In rat hepatoma cells, glucocorticoids induce the accumulation of mRNA, and thus augment the synthesis of tyrosine aminotransferase. In mouse lymphoma (S49) cell lines, steroids induce macromolecules that inhibit cell growth and ultimately lead to cell death. In both cell types, steroids penetrate the cell membrane and combine with specific cytoplasmic glucocorticoid receptor (GR) protein molecules that equilibrate between "active" and "inactive" conformations. Data indicate the existence of distinct classes of nuclear acceptor sites specific for different receptor-steroid complexes. The deoxyribonuclease sensitivity of the nuclear acceptor sites and the binding of soluble GR complexes to purified DNA indicate that DNA plays a role in the structure of GR acceptor activity. There does seem to be at least some element of specific protein-polynucleotide recognition, rather than simple ion exchange. It is suggested that the acceptor sites detected in cell-free experiments represent those involved in the biological action of the steroids and that in intact cells, more sites are available and they have a lower affinity for GR than corresponding sites in isolated nuclei. To aid in identifying nuclear acceptor sites, study of steroid action using steroid-resistant variants of S49 mouse lymphoma cells was performed. Three predicted resistant phenotypes were found: those with defective steroid binding activity, those retaining

receptor activity but where nuclear transfer of GR complexes does not occur (nt-), and those where both reactions occur but without cell death. Experiments with these phenotypes suggested that (1) the transition from steroid sensitive to resistant is the result of a mutation (at least in nt-), (2) formation of the receptor-steroid complex is necessary but not sufficient for hormone action and (3) the receptor has both hormone and nuclear binding sites. Results thus strongly suggest that DNA plays a major role in nuclear binding of receptor-glucocorticoid complexes. (18 refs)

179. LIVER CYTOSOL CORTICOSTEROID BINDER IB, A NEW BINDING PROTEIN.

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Fels Research Institute and Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

J Biol Chem; 250(17):6799-6805 1975

A new binding protein named corticosteroid Binder IB elutes just after ligandin in DEAE-Sephadex chromatograms. It has been partially purified to about 2500-fold over cytosol proteins. Calculation of the number of steroid binding sites, assuming one site per molecule of Binder IB fraction after DEAE-Sephadex chromatography, suggests a concentration of the binding protein of about 0.0004% of cytosol proteins. Its pI value is judged to be 7.5 to 8 from its elution position on DEAE-Sephadex chromatograms. IB has an apparent molecular weight of 30,500 plus or minus 10% by gel filtration and a Stokes radius of 20 A. Binder IB binds radioactive dexamethasone, cortisol, and corticosterone in vitro with estimated KD values of 1, 13, and 25 nM, respectively. Saturation curves are abnormal, showing two phases. The saturation curves within the physiological range of concentrations of steroid are abnormal and suggestive of cooperativity. The second phase, at concentrations of glucocorticoids above saturation and physiological levels, shows extensive binding. After fractionation from other steroid binding proteins, the specificity of binding from competition studies in vitro is dexamethasone greater than or equal to cortisol = corticosterone = estradiol-17beta greater than or equal to deoxycorticosterone = dihydrotestosterone greater than aldosterone = cortexolone greater than testosterone. Other steroids tested are less efficient ligands. The binding is probably noncovalent, but strong; and the complex becomes more dissociable as purification proceeds, suggesting a conformational change in the protein. Storage and rebinding with steroid are possible throughout the purification process, although extensive ligand dissociation and denaturation of the protein occur after the final purification step. Binding in vitro is temperature -sensitive and binding is sharply pH dependent with an optimum at 7.5. The ligand is the unmetabolized steroid as judged by extraction of steroid-IB complex with methylene chloride and subsequent thin layer chromatography. The physiological function of this protein is unknown at present and purification of the major corticosteroid hormone receptor to homogeneity may be required before the function of Binder IB is fully understood. (Author Abstract)

180. EFFECT OF CHEMICAL INACTIVATING AGENTS ON GLUCOCORTICOID RECEPTOR PROTEINS IN MOUSE AND HAMSTER CELLS (MEETING ABSTRACT).

Young HA, Parks WP, Scolnick EM

Natl. Cancer Inst., Bethesda, Md. 20014

Proc Natl Acad Sci USA; 72(8):3060-3064 1975

The effects of chemicals which inactivate the binding capacity of the cytoplasmic receptor protein for 3Hglucocorticoid hormones were studied. Treatment of mouse (cell line C3H MT Cl 101) or baby hamster kidney cell (BHK) (lacking thymidine kinase) cytosol with N-ethylmaleimide or iodoacetamide rapidly inactivated the 3Hglucocorticoid hormone binding activity of either cytosol. Prebinding the glucocorticoid hormone, dexamethasone, to the cytosol receptor blocked the rapid inactivation of the receptor by N-ethylmaleimide. Treatment of the bound hormone-receptor complex with iodoacetamide prevented the subsequent binding of the hormone-receptor complex to DNA without causing a dissociation of the complex. Although interpretation of the results is complicated because crude extracts were used, the results suggest that a sulfhydryl group is involved in the binding of glucocorticoid hormones to the receptor protein. It appears that iodoacetamide inactivates a separate chemical site which is necessary for the binding of the hormone-receptor complex to DNA.

181. THE REPRESSION AND DEREPRESSION OF HEPATIC TYROSINE AMINOTRANSFERASE BY CARCINOGENS.

Hanausek-Walaszek M, Schumm DE, Webb TE

Inst. Oncology, 44-100 Gliwice, Poland

Chem Biol Interact; 12(3/4):391-401 1976

A single carcinogenic dose of dimethylnitrosamine (50 mg/kg) initiated the induction cycle for hepatic tyrosine aminotransferase in adrenalectomized male Sprague-Dawley rats. Following this initial induction in the presence of dimethylnitrosamine, the enzyme became refractory to reinduction by known inducers. The administration of thioacetamide to either adrenalectomized or intact rats led to an immediate and progressive loss of inducibility by hydrocortisone, dibutyrylcyclic AMP or dimethylnitrosamine. Although the thioacetamide-induced repression was not reversed even ten weeks after cessation of treatment, it was reversed after the induction of liver regeneration. Both the carcinogen-mediated induction and repression of tyrosine aminotransferase appeared to occur by mechanisms not involving the corticosteroid-binding proteins that normally mediate the induction by glucocorticoids.

182. CORTICOSTEROID BINDING PROTEINS AND THE HORMONE RECEPTOR IN LIVER.

Litwack G

Fels Res. Inst., Dept. Biochemistry, Temple Univ. Sch. Medicine, Philadelphia, PA

Adv Enzyme Regul; 13:151-171 1975

The corticosteroid binding proteins and the hormone receptor in the liver were studied. The resolution of glucocorticoid binding proteins, after injection of hormone or after macromolecular binding was accomplished in vitro, was effected on Sephadex A-50 columns. Five protein fractions bound glucocorticoids and, in certain cases, bound some of their anionic molecules. The hormone receptor had a molecular wt of 67,000, which from gel filtration data was calculated to represent 35A The steroid-protein complex was recovered from the nucleoplasm after the nuclei were disrupted by freeze-thawing; the receptor had the same properties as the receptor isolated from cytoplasm, except that the molecular wt was apparently reduced from 67,000 to 62,000. The nucleoplasmic form was calculated to have a sedimentation constant of approx 4.0 S. A 7S-8S hormone receptor was visible only by labeling cytosol in vitro and not in vivo by the methods employed (gel filtration, Sephadex A-50, Sephadex G-100, and isoelectrofocusing). The present view is that either the 7-8S receptor has no physiological significance or it turns over very rapidly at 37 C. (34 refs)

183. PRETRANSLATIONAL CONTROL OF TRYPTOPHAN OXYGENASE LEVELS IN MORRIS HEPATOMA AND HOST LIVER.

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Cancer Res; 36(10):3594-3599 1976

Tryptophan oxygenase is present and hormonally inducible in host livers but is absent in transplanted Morris hepatomas examined under basal conditions as well as in hormonally induced animals. Studies were performed to determine whether the absence of tryptophan oxygenase in hepatomas is mediated by an alteration in the translational efficiency or the level of the messenger RNA (mRNA) for tryptophan oxygenase. The tissue level of the specific mRNA coding for tryptophan oxygenase was quantitated in an mRNA-dependent Krebs ascites cell-free protein-synthesizing system. The enzyme levels and mRNA activities in host livers and hepatomas from control rats and rats given injections of an inducing dose of hydrocortisone were compared; they indicate that the induction of tryptophan oxygenase in host livers by hormones is accompanied by a proportional increase in the level of its mRNA, whereas in the transplanted hepatomas the tryptophan oxygenase catalytic activity and the mRNA coding for this enzyme were undetectable in both control and glucocorticoid-induced animals. No functional mRNA for tryptophan oxygenase could be detected in the total polyadenylate-containing mRNA isolated from the Morris hepatoma cells. The hepatomas contained normal levels of cytoplasmic glucocorticoid receptor that could bind glucocorticoid, undergo "activation," and translocate to both normal and neoplastic nuclei. Thus, deletion of tryptophan oxygenase in hepatomas is a consequence of the absence of the gene product, ie, the tryptophan oxygenase mRNA, which codes for its synthesis; this is not due to detectable alterations in the ability of the glucocorticoid receptor to bind the steroid hormone, or of the hormone-receptor complex to undergo activation, or of the activated steroid-receptor complex to bind to nuclei derived from the hepatoma or normal liver. (Author Abstract)

184. PARTIAL PURIFICATION OF A GLUCOCORTICOSTEROID RECEPTOR FROM RAT LIVER CYTOSOL.

Govindan VM

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Hoppe Seylers Z Physiol Chem; 357(4):496 1976

We have attempted to purify from rat liver cytosol a receptor specific for natural and synthetic glucocorticosteroids. In addition to classical methods of separation of proteins, we have also applied affinity chromatography. To circumvent a disadvantage usually observed with the affinity techniques, ie the difficulty of eluting the receptor once it has been bound to the matrix, we have adopted the method of Sweet and Adair for our purpose. Accordingly a disulfide bridge between hormone and solid phase is constructed which is quite labile to SH reagents. Rat liver cytosol was prepared in a buffer consisting of 50mM KCl, 10mM Tris/HCl, pH 7.5 and 20% glycerol saturated with N2 at 0 - 4 degrees C. The cytosol was submitted to chromatography on Sephadex G-200, equilibrated with the same buffer. There was no loss of binding activity during this step. The exclusion peak containing the steroid receptor was incubated with the affinity adsorbent for 4 h, packed in a small column and washed thoroughly with buffer. The gel was then incubated with buffer containing 100mM mercaptoethanol for 4 h, resulting in the release of the receptor-hormone complex from the matrix. This single affinity step leads to a 6000-8000-fold purification of the receptor protein. (Author Abstract)

185. THE DEXAMETHASONE RECEPTOR IN THE NOVIKOFF HEPATOMA: CHARACTERIZATION AND CHANGES IN CONCENTRATION AND NUCLEAR UPTAKE DURING GLUCOCORTICOID THERAPY.

Liu SL, McNamara DJ, Webb TE

Dept. Physiol. Chem., Ohio State Univ., Coll. Med., Columbus

Biochem J; 152(3):689-695 1975

Novikoff hepatoma ascites cells, grown ip in rats, are shown to possess a high-affinity, low-capacity dexamethasone-binding receptor protein with properties similar to the G protein (dexamethasone-binding protein) of normal rat liver. Tumor cells were maintained in female Sprague-Dawley rats and the receptor protein was identified by modified steroid-binding assay of the cytosol fraction upon differential centrifugation following 2-hr in vitro incubation to deplete the cells of endogenous glucocorticoids. After 1-hr incubation of tumor cells in medium containing 100 nM ^{3}H-dexamethasone, approx. 50% of radioactivity was located in the nucleus and only 0.7% existed in bound form in the cytosol. Kinetic studies revealed that binding was completed within 2 hr and near saturation of the receptor was attained at a dexamethasone concn of 80 nM. The association constant (Ka) was 1.3 x 10^8 M**-1 with dexamethasone, and the receptor site concn was estimated to be 1.7 x 10**-9 M when tumor-bearing rats were treated with cortisone acetate (25 mg/kg/d i.p on d. 2, 4, and 6 of each weekly transplant generation), changes occurred in the binding of dexamethasone by receptor protein and of dexamethasone-receptor complex by isolated nuclei, accompanied by alteration of the association constant for the dexamethasone-receptor complex. After 12 generations the receptor protein concn had stabilized at 50% of the control value, while marked cyclic increases occurred in the nuclear uptake of receptor-bound labeled glucocorticoid over 4 generations. Tumor cells appeared to exhibit significant resistance to therapeutic doses of cortisone acetate (75 mg/kg) after 7 or 12 generations of treatment with low doses, and it was suggested that clonal-type selection had occurred in that the DNA content in generations 8 and 13 was twice that of the sensitive line carried in untreated animals or that measured in generation 4. (23 refs)

186. THE EFFECT OF HYDROCORTISONE ON THE RNA SYNTHESIS IN CELL NUCLEI OF RAT LIVER AND ZAJDELA ASCITES HEPATOMA.

Adler VV, Shapot VS, Shittabe NK, Volchek AG, Rozen VB

Inst. Experimental and Clinical Oncology, Acad. Medical Science, Moscow, USSR

Vopr Onkol; 21(7):80-86 1975

The activation of RNA synthesis in the nuclei of normal rat liver cells, of Zajdela ascites hepatoma cells, and of liver cells of tumor-bearing rats after intraabdominal administration of 5 mg/100 g of hydrocortisone was studied. White male rats, weighing 150-200 g, received 41 Ci/mM of ^{3}H-hydrocortisone 4-6 days after adrenalectomy. Isolation of rat liver cells and of ascites hepatoma cells was carried out at 4 C. Determination of activity in the RNA-synthesizing system of the nuclei was performed with an incubation mixture of tris-HCl, MgCl2 , beta-mercaptoethanol, various triphosphates in micromols, and a radioactive precursor. Each incubated 0.25-ml sample contained cell nuclei expressed in terms of DNA at varying quantities. In vitro administration of hydrocortisone stimulated RNA synthesis in the normal liver cells of adrenalectomized rats. This effect was absent in the nuclei of Zajdela ascites hepatoma cells. Injection of the hormone into

animals with hepatoma stimulated RNA synthesis in liver cell nuclei much less than in the control animals. The results of the in vitro experiments were likely to be due to a disorder in the hepatoma cells affecting some link in the mechanism of hormonal regulation. The absence of specific protein receptors in the animals with ascites tumor would be the most probable reason for the inability of the hepatoma cell to react to the hormone, which could be caused by a reduced concentration of receptors in the cytoplasm of the hepatocytes. The results characterize phenotypic, rather than genetic, peculiarities of normal and tumor cells.

187. GLUCOCORTICOID CYTORECEPTORS IN NORMAL AND TUMOR TISSUES OF RAT LIVER.

Shittabe K, Volchek AG, Adler VV, Rosen VB
Biol. Fac., Moscow Univ., USSR
Vopr Med Khim; 20(6):625-631 1974

Studies were carried out on the livers of random-bred albino rats with Zajdela ascites hepatoma to investigate the cytoplasmatic and nuclear receptors of the tumor; cell receptors of the rat liver with ascites hepatoma; and the main binding parameters of receptor proteins in the cytoplasm and nucleus of the liver of intact rats. The cytoplasm of intact rat liver has a corticosteroid-binding system of three macromolecular components with association constants of the order of 1011, 108 and 107 M-1, resp. The first two components were assumed to be cytoplasmic receptors of the liver cell as they demonstrated high specific affinity for physiologically active corticosteroids; the concentration of their binding groups corresponded to physiological concentrations of glucocorticoids in the cells. The third component was shown to be a nonspecific binding factor. The liver cell nuclei contained only two macromolecular components with association constants of 1011 and less than 107 M-1. It is suggested that the former component is a nuclear receptor of the liver cell, while the latter is a nonspecific binding factor. No specific receptor macromolecules were found in either the hyaloplasm or nuclei of Zajdela ascites hepatoma cells, suggesting that these malignant cells have become resistant to glucocorticoids. In addition, no cytoplasmic or nuclear receptors were detected in the livers of rats with Zajdela ascites hepatoma; only a nonspecific binding factor was present. It is suggested that the disappearance of glucocorticoid receptors and possibly the hormone resistance of the liver may be due to a toxic effect produced by the rapidly developing ascites hepatoma which killed rats 10 days after transplantation.

188. COMPARISON OF GLUCOCORTICOID RECEPTORS IN CYTOSOLS OF NORMAL LIVER, LIVER OF TUMOR-BEARING ORGANISM, AND ZAJDELA HEPATOMA.

Dmitrieva LV, Volchek AG, Rozen VB, Adler VV, Shapot VS
Lab. Endocrinology, Biology Faculty, M. V. Lomonosov State Univ., Moscow, USSR
Biochem J; 41(10):1502-1508 1976

Specific protein receptors for two glucocorticoids, dexamethasone (D) and hydrocortisone (HC) were observed in the cytosols of cells of normal liver, hormone-resistant Zajdela ascites hepatoma, and the livers of tumor-bearing animals. Male rats of the Wistar line were utilized. A study of the competition of unlabeled hormones for the binding of ^{3}H-D by the cell receptors of the livers of intact animals showed that the receptors had significant hormonal stereospecificity. The receptors of liver cell cytosol had an association constant of 3.8 X 10^8 M**-1 for D and 0.57 X 10^8 M**-1 for HC, as well as a limited number of steroid-binding sites. The steroid-receptor complexes of the cytosols of liver cells and ascites hepatoma sedimented in the 6S-7S region in a buffer of low ionic strength and in the 3S-4S region in a buffer of high ionic strength. The affinity of the receptors for HC and D changed as the Zajdela ascites hepatoma grew, and the number of steroid-binding sites declined three- to fourfold compared to normal liver. This can partially explain the resistance of the hepatoma to glucocorticoids. (25 Refs)

189. ELEVATED CONCENTRATION OF A DEXAMETHASONE-RECEPTOR TRANSLOCATION INHIBITOR IN NOVIKOFF HEPATOMA CELLS.

Liu SL, Webb TE
Dept of Physiological Chemistry, Ohio State Univ Coll of Medicine, Columbus, OH 43210
Cancer Res; 37(6):1763-1767 1977

Despite the apparent similarity of the dexamethasone-receptor concentration in the rat liver and Novikoff hepatoma cytoplasms, the in vitro translocation of the dexamethasone-receptor complex into liver or hepatoma nuclei was three times greater from liver as compared to hepatoma cytosol; both cytosols showed an apparent saturation of nuclear receptor sites. Although the competitive interaction of the receptor complex with nuclei suggested that they were qualitatively similar, the translocation of additional dexamethasone-receptor from liver cytosol into nuclei presaturated with the receptor complex from hepatoma cytosol suggested that they were different. However, the latter observation, as well as the initial differential nuclear uptake of the dexamethasone-receptor complex from liver and hepatoma cytosols, can be accounted for by a higher concentration of a translocation inhibitor in the hepatoma cytosol. Thus hepatoma cytosol, at a protein concentration of 6 mg/ml and free of activated receptor complex, was four times more inhibitory to nuclear uptake of the activated complex than was a comparable preparation of liver cytosol. A study of the kinetics of nuclear uptake of the partially purified dexamethasone-receptor complex from hepatoma confirmed that, in the absence of cytoplasmic translocation inhibitor, nuclear acceptor sites were not limiting in vitro. Evidence is presented which suggests, but does not prove, that the cytoplasmic translocation inhibitor functions as such in the intact cell. (23 Refs) (Author Abstract)

190. GLUCOCORTICOID REVERSAL OF TRANSFORMED MEMBRANE PHENOTYPE IN HEPATOMA CELLS (MEETING ABSTRACT).

Carlson SA, Gelehrter TD
Depts. Human Genetics and Internal Medicine, Univ. Michigan, Ann Arbor, MI 48109
J Cell Biol; 70(2/Part2):18a 1976

Neoplastic transformation is associated with structural and functional alterations in the cell membrane which may have an important role in growth regulation. Hormones can also affect membrane phenotype in a manner similar to neoplastic transformation. In HTC cells, an established line of rat hepatoma cells in tissue culture, glucocorticoids reverse several membrane properties characteristic of transformed cells, but do not alter the growth rate of the cells. Dexamethasone (Dex), a synthetic glucocorticoid, rapidly and reversibly inhibits the rate of influx of selected amino acids by a process requiring concomitant protein synthesis. In addition, Dex decreases the number of microvilli on the surface of HTC cells in suspension culture, as assessed by scanning electron microscopy; and has been reported to increase the adhesiveness of HTC cells, and to decrease the production of plasminogen activator, a membrane-associated protease. Using an agar-fibrin overlay technique to detect plasminogen activator production by individual colonies of HTC cells, we have selected lines of HTC cells resistant to the inhibitory effect of dexamethasone. These variant cell lines will be tested for the ability of Dex to induce tyrosine aminotransferase;

normal induction would indicate normal glucocorticoid receptor function. The variants will then be tested to determine whether the presumed mutations are pleiotropic. The analysis of several such variant lines by both biochemical and genetic techniques should facilitate the study of hormonal regulation of membrane phenotype.

191. GLUCOCORTICOID CONTROL OF GENE EXPRESSION. (PP. 25-35)

Kenney FT

Control Mechanisms of Cancer, Progress in Cancer Research and Therapy. Criss WE, Ono T, Sabine JR, ed. New York, Raven Press, Vol. 1, 1976.

The concept that glucocorticoids (GC) function at the nuclear level to control the expression of specific genes is discussed. Experiments concerning the induction of tyrosine aminotransferase (TAT) in liver or hepatoma cells and leukemia virus activation on mouse cells are also reviewed. GC promote specific transcriptions, since synthesis of specific proteins is increased by these hormones, messenger RNA coding for these proteins accumulates in GC-treated cells, synthesis of RNA is required, and the steroid must interact with the site of RNA synthesis before induced protein synthesis begins. Three Morris hepatomas were studied to determine if loss of sensitivity to hormonal control in tumors is due to loss of the receptor protein. The hepatomas were 7800, in which TAT is present but cannot be induced by GC. Glucose gradient analysis showed that all three contained readily detectable amounts of GC receptor. The binding capacity of receptors from each of the hepatomas was similar to that of the liver receptor, and all of the receptors were translocated to liver nuclei with comparable efficiency. Therefore, the nonresponsiveness of hepatomas 9633 and 9618A with GC cannot be attributed to a defect in the receptor system. Leukemia virus activation in mouse AKR cells by iododeoxyuridine (IUdR) was dissociated into two phases: incorporation of IUdR into DNA and synthesis of viral protein. Hydrocortisone had no effect when added in the first stage but was fully effective when added in the second. Hydrocortisone cannot replace IUdR as activator. Therefore, the steroid clearly modulates the expression of genes activated by IUdR substitution into DNA. (27 Refs)

192. REGULATION OF GENE EXPRESSION.

Tomkins G

no affiliation given

Cancer Biology; I. Induction, Regulation, Immunology and Therapy. Based on a Series of Lectures Presented at the Given Institute of Pathobiology in Aspen, Colorado, July 1974. Edited by R.R. Pascal, F. Silva and D.W. King. (New York: Stratton Intercontinental Medical Book Corp.):24-28 1976

The role of glucocorticoids and cyclic AMP in the regulation of cell replication is discussed. In cultured rat hepatoma cells at 0 C, glucocorticoid steroids (not specified) penetrate the cell membranes and react with intracellular receptor proteins. The receptor:glucocorticoid complex, after undergoing further activation, develops a strong affinity for and attaches to nuclear acceptor sites containing DNA. The synthesis of 5-10 specific proteins in cultured hepatoma cells is activated by glucocorticoids. There is a good correlation between the rate of synthesis of one of these proteins, tyrosine amino transferase, and the appearance of receptor in the nucleus. The steroids do not affect enucleated cells. Because of these observations, the site of the complex is thought to lie within the nucleus. The addition of glucocorticoids to S49 (T lymphoma cells of the mouse) blocks the growth of some cells; ultimately they die. The resistant mutant cells (arising spontaneously at $1/10^6$ cells/generation) can thus be selected. Study of the phenotypes of these resistant mutants indicates that the receptor has three domains: a nuclear DNA receptor, a steroid receptor, and an inhibitory portion. Glucocorticoids could inhibit cell growth and kill neoplastic cells (such as lymphomas) by inducing adenyl cyclase or repressing the phosphodiesterase, leading to an increase in cyclic AMP, which in turn would induce a block in G1. S49 cyclic-AMP-resistant cells have been isolated which are missing the binding protein (cyclic nucleotide receptors) and protein kinase but which are still responsive to steroid inhibition, indicating that growth arrest by glucocorticoids is not mediated by cyclic-AMP. Transformed cells appear to have difficulty in responding to the normal signals which initiate growth arrest although the mechanism for the transition appears intact.

193. EVIDENCE FOR A PHOSPHOLIPID REQUIREMENT IN THE SPECIFIC BINDING OF GLUCOCORTICOIDS TO RECEPTORS OF FIBROBLASTS AND THYMIC LYMPHOCYTES.

Schulte HF, Neilsen CJ, Sando JJ, Pratt WB

Department of Pharmacology, The University of Michigan School of Medicine, Ann Arbor, Michigan 48104

J Biol Chem; 251(8):2279-2289 1976

The specific steroid binding capacity of soluble preparations from mouse fibroblasts and rat thymic lymphocytes in inactivated by incubation with phospholipases. Receptor binding is drastically reduced by very low concentrations of boiled phospholipase A preparations from bee venom and snake venoms. The enzyme effect is calcium-dependent and is blocked by both phospholipid and a substrate analog that is a competitive inhibitor of phospholipase A. The specific binding capacity is also sensitive to digestion by phospholipase C. Two possible mechanisms are considered for the phospholipase A effect: (a) the receptor protein may be associated with a phospholipid component which is required for specific hormone binding; (b) phospholipase A may be producing detergent products that are indirectly inactivating the receptor. Examination of the effects of lysophosphatide on the receptor and assay of lipid phosphate in the receptor preparation do not support a mechanism based solely on detergent effects. Because phospholipase C, which does not produce detergent products, also inactivates the binding, we propose that the phospholipases may be digesting phospholipid which is a requisite component of the glucocorticoid receptor. (Author Abstract)

194. CONTROL OF STEROID RESPONSIVENESS IN CULTURED LYMPHOID CELLS.

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Hoppe Seylers Z Physiol Chem; 357(4):496-497 1976

Glucocorticoids exert a striking effect on certain types of lymphoid cells resulting in the inhibition of active membrane transport, the inhibition of cell proliferation, and finally cell lysis. These effects can be observed both in normal and in neoplastic lymph cells which in the case of mouse lymphoma and myeloma cells can be propagated in continuous culture. For the investigation of steroid hormone action such a system offers the great advantage that mutant (or variant) cells with altered responses to the hormone can be isolated by selection and cloning. Biochemical studies on the mode of action of steroid hormones strongly suggest a chain of early events which include the binding of the steroid to specific cytoplasmic binding proteins (receptors), temperature dependent activation of the receptor-hormone complex, and the association of the complex with DNA containing sites in the nucleus of a responsive cell. These early events which appear to occur in all steroid responsive systems are followed by cell and hormone specific processes. In accordance with this

general scheme three different types of glucocorticoid resistant lymphoid cells have been identified thus far. In some of these resistant cells the receptor-steroid complex has been shown to possess increased binding to DNA. The technique of somatic cell hybridization has been applied to two lymphoma cell lines and one myeloma line. Dominance tests were carried out in wild type x resistant combinations and the resulting hybrids proved to be steroid sensitive (although to a lesser extent than the wild type cells). In another series of experiments hybrids between various resistant cells were prepared in an attempt to complement the resistance due to different types of defects. These hybrid cells were steroid unresponsive and apparently did not contain hybrid receptor molecules. Glucocorticoid receptors - and steroid hormone receptors in general - are proteins with two different active sites, one involved in steroid binding and the other in nuclear association. Several lines of evidence indicate that these two sites interact with each other and the present experiments suggest that they are within the same complementation group. (Author Abstract)

195. STUDIES ON GLUCOCORTICOID-INDUCED CYTOLYSIS IN CULTURED MOUSE LYMPHOMA CELLS, L5178Y. I. GLUCOCORTICOID SPECIFICITY AND CYTOPLASMIC RECEPTORS IN SENSITIVE AND RESISTANT CELLS.

Kondo H, Kikuta A, Noumura T
Dept. Biol., Tokyo Metropolitan Inst. Gerontol., Tokyo, Japan
Exp Cell Res; 90(2):285-297 1975

A study of glucocorticoid-induced cytolysis of L5178Y lymphoma cells, on a selection of hormone-resistant cells from a sensitive parent cell line, and on a comparison of glucocorticoid binding to both cell types is reported. Dexamethasone (D) at 2 x 10**-8 M induced only a few cytolytic cells, but cells continued to grow at 2 x 10**-7 M, during 24 hr exposures. After 48 hr, cytolytic cells were much increased. Dexamethasone concn higher than 2 x 10**-6 M resulted in more cytolysis, in proportion to the concn. At 2 x 10**-4 M, some cells still survived. Dexamethasone-induced cytolysis characteristically appears later than 24 hr after addition of the agent. The sensitive cell line that was evolved showed no inhibition of growth at 2 x 10**-6 M dexamethasone. The parent line was almost completely lysed at 2 x 10**-5 M. The specific uptake curve for dexamethasone in resistant cells was about half that of sensitive cells. A significant part of the drug was associated with the nuclear fraction of both cell types. The cytoplasm of sensitive cells contained about 2.5-fold as many binding sites (5.7 x 10**-10 mmol/mg protein) as that of resistant (2.3 x 10**-10 mmol/mg protein) cells. Binding was glucocorticoid-specific in both cell types. Dexamethasone would bind to cell nuclei only when complexed with a cytosol receptor in both cell types. These studies suggested an equal level of nuclear association sites for cytoplasmic steroid-receptor complex in the two cell types. (24 refs)

196. THE ROLE OF SH-GROUPS IN THE INTERACTION OF STEROIDS WITH THE CYTOPLASMIC GLUCOCORTICOID RECEPTOR FROM RAT THYMUS.

Rees AM, Bell PA
Welsh Natl. Sch. Medicine, Heath, Cardiff, CF4 4XX, Wales
J Endocrinol; 6:(3):4-5 1975

The cytoplasmic glucocorticoid receptor protein of rat thymus, and especially the stabilization by bound hormone, was studied. The specific, saturable binding of 1,2**-3 Hdexamethasone or 1,2,4**-3 Htriamcinolone acetonide to a postmicrosomal supernatant fraction of thymus tissue from adrenalectomized rats was measured at 4 C using a dextran-charcoal competitive binding assay. When the supernatant was incubated at 4 C for various times before the addition of 1,2**-3 Hdexamethasone, the maximum amount of steroid specifically bound by the receptor was found to decline exponentially with pre-incubation time, with a half-life of about 2 hr. Addition of 2-mercaptoethanol (20 mM) or dithiothreitol (5 mM) slowed but did not completely stop the decline. The receptor was also stabilized by EDTA (2.4 mM/liter), an effect which was additive with that of the thiol, and smaller. Delayed addition of EDTA and dithiothreitol resulted in recovery of steroid binding to the level obtained when the protecting agents were present from time 0. The effect of N-ethylmaleimide (NEM) on the binding of 1,2,4**-3 Htriamcinolone acetonide to a thymus supernatant fraction of 4 C was studied. Addition of NEM (0.5 or 5 mM/liter) before or with the steroid abolished all specific binding. Delayed addition of 0.5 mM NEM prevented further binding, and delayed addition of 5mM NEM caused dissociation of previously-formed complex. In experiments using dexamethasone, the higher concentration of NEM caused a greater loss of bound steroid than did the addition of excess nonradioactive steroid. Similar results were obtained with p-chloromercuriphenyl sulfonic acid.

197. THE INVOLVEMENT OF RECEPTOR SULPHYDRYL GROUPS IN THE BINDING OF STEROIDS TO THE CYTOPLASMIC GLUCOCORTICOID RECEPTOR FROM RAT THYMUS.

Rees AM, Bell PA
Welsh Natl. Sch. Medicine, The Heath, Cardiff, Wales
Biochim Biophys Acta; 411(1):121-132 1975

Binding the steroids, dexamethasone, 9alpha-fluoro-16alpha-methyl-11beta,17,21-trihydroxypregna-1,4-diene-3,20-dione, or (triamcinolone acetonide, 11beta,21-dihydroxy-9alpha-fluoro-pregna-1,4-diene-3,20-dione-16alpha, 17alpha-acetonide, was studied using thymus tissue from 8-12 wk-old rats adrenalectomized 1 or 2 wk previously. The glucocorticoid receptor protein present in the supernatant fraction of rat thymus tissue has a half-life of about 2 hr at 4C. The decline in steroid-binding capacity could be slowed, by the addition of sulphydryl-protecting agents such as 2-mercaptoethanol or dithiothreitol, and by EDTA. The inactivation was also partly reversed by these agents. 0.5 mM N-ethylmaleimide or p-chloromercuriphenylsulphonic acid inactivated the receptor at 4C, but bound steroid (1,2,4' H) triamcinolone acetonide protected the receptor against this inactivation. Bound steroid did not protect the receptor against the action of higher concentrations of these reagents. Treatment of intact thymus cells with 2,4-dinitrophenol at 1 mM prevented detectable binding of steroid by the supernatant fraction from these cells. If 5 mM dithiothreitol was present during extraction, 1.59 picomoles/mg protein of dexamethasone was bound compared to 4.03 picomoles bound by the control.

198. ATP, CYCLIC AMP AND THE REGULATION OF CORTICOSTEROID BINDING BY RAT THYMUS CELLS IN VITRO (MEETING ABSTRACT).

Sloman JC, Bell PA
Tenovus Inst. Cancer Res., The Heath, Cardiff, CF4 4XX, Wales
J Endocrinol; 67(2):27P 1975

The energy-dependence of corticosteroid binding to receptors in the nuclei of intact rat thymus cells was studied in vitro using a rapid, sensitive filter assay. It was concluded that in the absence of ATP, the corticosteroid receptor protein is present either in an inactive form or in a location inaccessible to the steroid, and that its regeneration or availability is dependent on ATP.

199. WHY CORTICOIDS ARE EFFECTIVE AGAINST A WIDE VARIETY OF CANCERS.

Lipsett MB

Natl. Inst. Child Health Human Dev., Natl. Inst. Health, Bethesda, MD

Ann NY Acad Sci; 230:489-490 1974

Recent studies on ways in which steroid hormones exert their effects on cells are briefly summarized; these include studies on: specific receptors for glucocorticoids in hepatoma, the specificity of the cytosol binding protein, the possible role of glucocorticoid receptors in P-1798 lymphosarcoma cells and mouse lymphoma cells, and effects on antibody production and cell-mediated immunity. (56 refs)

200. GLUCOCORTICOID RECEPTORS IN LYMPHOID TISSUES.

Rosen F, Kaiser N, Turnell RW, Milholland RJ

Dept. Therapeutics, Roswell Park Mem. Inst., Buffalo, N.Y.

Perspectives in Cancer Research and Treatment; Edited by GP Murphy, D Pressman, EA Mirand (New York, Alan R Liss, Inc):355-363 1973

Studies on the biochemical basis for the action of steroid hormones on lymphoid tissues are reviewed. The presence of specific glucocorticoid receptors has been demonstrated in thymocytes, lymphoid cells, HeLa cells, cultured fibroblasts, and P1798 cells, and the binding of ^{3}H-triamcinolone acetonide was investigated in rat and mouse thymocytes and P1798 cells. Sedimentation profiles of the steroid-receptor complex on sucrose gradients are consistent with a temperature-dependent translocation from cytoplasm to nucleus. Cortexolone (11-deoxycortisol) inhibits the binding of triamcinolone acetonide in rat thymocytes by 90%, blocking steroid-induced inhibition of 2-deoxyglucose transport and forming a nondissociable complex with the receptor protein. Preliminary studies have indicated that cortexolone is inactive as an antagonist of hepatic enzyme induction and thymic involution, but the development of antisteroids, which will inhibit the growth of steroid hormone-dependent tumors (such as breast, ovarian, uterine, and prostatic cancer), remains of considerable practical importance. (28 refs)

201. GLUCOCORTICOIDS: RECEPTORS AND MECHANISM OF ACTION IN LYMPHOID TISSUES AND MUSCLE.

Rosen F, Kaiser N, Mayer M, Milholland RJ

Dept. Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Inst., New York State Dept. Health, Buffalo, NY

Methods Cancer Res; 13:67-100 1976

The receptors of glucocorticoids and their mechanism of action in muscle and lymphoid tissues are described. The physiological and biochemical action of these hormones on target tissues involves their binding specifically to cytoplasmic protein receptors. A hormone-resistant lymphoid tumor grown in culture has been observed to be deficient in the number of glucocorticoid-binding receptor molecules in comparison to the sensitive line. As a result, there has been much interest in the development of antisteroids that might be utilized in the treatment of hormone-dependent neoplasms. There have also been investigations into modalities of treatment that might depress or stimulate the biosynthesis of the receptor molecule or that might block the nuclear activity of the hormone-receptor complex. Further elucidation of the mode of action of glucocorticoids may permit their effective use in the treatment of neoplasms. (114 refs)

202. THE CELLULAR ACTIONS OF GLUCOCORTICOIDS IN RELATION TO HUMAN NEOPLASMS. (PP. 114-132)

Thompson EB

Steroid Hormone Action and Cancer. Menon KM, Reel JR, ed. New York, Plenum Press, Current Topics in Molecular Endocrinology, vol. 4, 1976.

In relation to human neoplasms, the cellular actions of glucocorticoids are described. Specific glucocorticoid receptors were observed to exist in the lymphoblasts of some patients with acute lymphoblastic leukemia and not in others; these receptors were apparently necessary for active steroids to exert their inhibitory effects on the cells in vitro. The low levels of binding in the resistant cells may be due to an altered receptor with lower steroid affinity, a lowered receptor content in all cells, or a small subpopulation of cells with a complete complement of receptors. It is concluded that the investigation of steroid receptors may provide a clue to the mechanisms by which leukemias escape from steroid responsiveness and that the measurement of the receptors may be a means of predicting the in-vivo response to hormonal therapy. (64 refs)

203. GLUCOCORTICOID BINDING PROTEINS IN LEUKEMIC CELLS.

Thompson EB, Lippman ME

Natl. Inst. Health, Bethesda, Md.

Proc Am Assoc Cancer Res; 15:121 1974

CORTICOSTEROIDS (General and Unspecified): binding to proteins; LEUKEMIA: lymphoblastic, human, cells; PROTEINS: binding of corticosteroids.

204. CYTOPLASMIC GLUCOCORTICOID-BINDING PROTEINS IN GLUCOCORTICOID-UNRESPONSIVE HUMAN AND MOUSE LEUKEMIC CELL LINES.

Lippman ME, Perry S, Thompson EB

Natl. Cancer Inst., Bethesda, Md.

Cancer Res; 34(7):1572-1576 1974

Cytoplasmic glucocorticoid-binding proteins of high affinity and specificity were identified, quantified, and partially characterized in three steroid-unresponsive tissue culture lines, one derived from an AKR mouse leukemia and two derived from human lymphoblastic leukemia cells. The number of receptors/cell appeared to be comparable to that found in many steroid-responsive cells. Temperature-dependent entry of cytoplasmic receptor into the nucleus did not appear to be abnormal as determined in an in vitro nuclear binding assay. Cell growth, nucleoside and amino acid incorporation into macromolecules, amino acid pools, and glucose uptake were found to be unaffected by added glucocorticoid. The presence of specific cytoplasmic receptor proteins for glucocorticoids in malignant tissue does not appear to guarantee steroid responsiveness.

205. GLUCOCORTICOID BINDING PROTEINS IN MYELOBLASTS OF ACUTE MYELOGENOUS LEUKEMIA.

Lippman ME, Perry S, Thompson EB

Natl. Cancer Inst., Bethesda, Md.

Am J Med; 59(2):224-227 1975

The presence of specific cytoplasmic steroid binding protein (SBP) in leukemic myeloblasts was investigated, and whether their presence correlated with hormone responsiveness in vitro. Myeloblasts were obtained from 16 untreated children with acute myelogenous leukemia and assayed for the presence of SBP with either activated charcoal or filtration techniques. SBP was detected in myeloblast cytosol preparations of 3/16 pts. The presence of glucocorticoid binding protein activity was positively correlated with

inhibition of macromolecular synthesis of myeloblasts in vitro, whereas absence of SBP was associated with lack of glucocorticoid inhibition of the cells. All 3 pts with SBP responded by achieving a complete or partial remission to a drug combination containing glucocorticoid. Further studies are recommended to determine whether a correlation exists between SBP content and in vitro and clinical responses to steroid therapy in pts with acute myelogenous leukemia. (17 refs)

206. EFFECT OF GLUCOCORTICOIDS ON ACTIVATION ON LEUKEMIA VIRUS IN AKR MOUSE EMBRYO CELLS.

Ihle JN, Lane SE, Kenney FT, Farrelly JG
Oak Ridge Natl. Lab., Tenn.
Cancer Res; 35(2):442-446 1975

The effect of glucocorticoids on activation and replication of leukemia virus in AKR mouse embryo cells was analyzed. Using AKR mouse embryo cells, passage 44, activation of endogenous AKR virus was assayed by immunofluorescent techniques. An affinity chromatography method was used to distinguish intracellular viral type reverse transcriptase from cellular DNA polynucleases. In preliminary experiments using immunofluorescent techniques to determine glucocorticoid effects of virus activation, an optimal concentration of 0.5-1 microM hydrocortisone increased the number of cells scored as positive by 2 to 3-fold; there was no effect of hydrocortisone on incorporation of 3Hleucine into the cellular proteins. Phase-specific action of hydrocortisone indicated that only when hydrocortisone was added during the second phase was virus protein synthesis enhanced; the results indicate that hydrocortisone does not affect formation of the activation intermediate, but modulates the extent of expression after activation. The effect of hydrocortisone on chronically infected AKR cells was to approximately double the virus yield. In contrast, no change was observed in intracellular reverse transcriptase activity. Studies on a dexamethasone-binding protein suggest the presence of a single class of receptor sites; the protein nature of such 4S receptors is indicated by the finding that the 3Hdexamethasone complex is not sensitive to DNase or RNase, but completely destroyed by pronase. The observations suggest that the intracellular level of the structural virus proteins are probably increased by the hormone, and that the reverse transcriptase may be the limiting component for formation of complete virus.

207. CONDITIONS FOR OPTIMAL MTV SYNTHESIS IN VITRO AND THE EFFECT OF STEROID HORMONES ON VIRUS PRODUCTION.

Dickson C, Haslam S, Nandi S
Dept. Zool., Univ. California, Berkeley
Virology; 62(1):242-252 1974

The DNA polymerase assay was used with synthetic template/primers to measure the synthesis of mouse mammary tumor virus (MTV) obtained from the tissue culture fluid of primary mouse mammary tumor cells in vitro. A high level of DNA polymerase activity was obtained with the synthetic template/primer rA:dT10 and a low level was obtained with dA:dT10. The DNA polymerase activity was associated with particles exhibiting the physical, morphological, and immunological properties of MTV; there was a linear relationship to protein concentration up to 40 microg/ml. A high cell seeding density was required for optimal MTV synthesis, and synthesis was stimulated by a variety of steroid hormones; dexamethasone was the most potent, followed by hydrocortisone, corticosterone, aldosterone, and deoxycorticosterone. In the presence of fetal calf serum, insulin had a slight stimulatory effect. An increase in the rate of MTV synthesis was detectable 8-10 hr after exposure of the cells to hydrocortisone or other steroid hormone systems; these systems had no detectable effects on the overall rate of DNA, RNA, or protein synthesis. The results suggest that the rate of MTV synthesis is controlled via a receptor mechanism acting either indirectly on a critical cell function required for MTV synthesis or directly on an integrated viral genome.

208. ON THE EFFECTS AND THE MECHANISM OF ACTION OF CORTICOSTEROIDS IN NORMAL AND NEOPLASTIC TARGET TISSUES: FINDINGS AND HYPOTHESES: WITH A REVIEW OF INFORMATION ON INTRACELLULAR STEROID RECEPTORS IN GENERAL.

Kornel L
Rush-Presbyterian-St. Luke's Med. Cent., Chicago, Ill.
Acta Endocrinol Suppl (Kbh); 74(178):5-45 1973

Corticosteroids and intracellular steroid receptors in general are discussed. Information is included on glucocorticoids: metabolic effects, target tissues, induction of malignant tumors, effect on spontaneous neoplasms and hepatoma cells, and structural relationship to activity. Intracellular steroid receptors are considered in terms of evidence for their existence, characterization, binding, physiology, cytoplasmic and nuclear steroid-receptors, steroid-chromosomal protein interactions, chromosomal proteins and RNA synthesis, and gene depression in normal and neoplastic tissues. A hypothesis is presented of the mechanism for the opposing effects of glucocorticoids on liver vs lymphoid tissues. Glucocorticoid receptors and transcortin are also discussed, as well as evidence for a repressed lympholytic mechanism in chronic lymphocytic leukemia lymphocytes and its possible relationship to deranged steroid binding. (267 refs)

II. CYCLIC AMP AND CYCLIC GMP BINDING PROTEINS

A. Characterization of Cyclic Nucleotide Binding Proteins and Their Roles in Protein Kinase Systems

209. PROTEIN KINASE ACTIVITY IN MOUSE MAMMARY CARCINOMA.

Majumder GC
Indian Inst. Experimental Medicine, Calcutta-32, India
Biochem Biophys Res Commun; 74(3):1140-1145 1977

The protein kinase activity of mouse (C3H BA) mammary adenocarcinoma was studied. The specific activities of cyclic AMP-dependent (RC) and independent (C) protein kinases and a specific cyclic AMP-binding protein (R) were markedly lower in carcinoma (0.0045, 0.013, and 6.0 units/mg DNA, respectively) than they were in normal mammary cells (0.0162, 0.0682, and 17.1 units/mg DNA, respectively). Mammary carcinoma also showed much lower (1.5 units/mg DNA) specific activity of the cyclic AMP-binding protein than did normal cells (3.4 units/mg DNA), indicating a lower dependency of protein kinase activity on cyclic AMP during neoplasia. Like normal mammary gland cells, carcinoma cells showed the presence of two protein kinase peaks (I and II), as resolved by diethylaminoethyl-cellulose chromatography. The activity of protein kinase I was independent of cyclic AMP, whereas kinase II was markedly stimulated (about five-fold) by 1 muM cyclic AMP. Chromatography profiles of the protein kinases differed with respect to normal and carcinoma cells. Kinase I and II represented about 25% and 75%, respectively, of the total activity in normal tissue and about 50% each in malignant tissue. Specific activities of kinase I and II were higher in normal cells than in carcinoma by about two- and six-fold, respectively. Thus, malignancy of the mammary gland is associated with a marked increase in the ratio of cyclic AMP-independent to dependent protein kinase. Studies with

various cyclic nucleotides demonstrated that the neoplastic mammary protein kinase II, like the normal mammary enzyme, is also activated specifically by cyclic AMP. Kinase I appears to represent the catalytic subunit (C) which can be converted to the cyclic AMP-dependent form (RC) after interaction with the specific cyclic AMP-binding protein (R). The lower responsiveness to cyclic AMP for the activation of carcinoma protein kinase may thus be attributed to a higher ratio of C/RC in carcinoma than in normal cells. Altering the cyclic AMP responsiveness in the mammary carcinoma may cause a major change in the normal regulatory mechanisms in these cells. (12 Refs)

210. ALTERED INTERACTION BETWEEN BINDING AND CATALYTIC SUBUNITS OF A CYCLIC AMP-STIMULATED PROTEIN KINASE FROM HEPATOMA CELLS.

Mackenzie CW, Stellwagen RH
Dept. Pharmacology, Univ. Minnesota, Minneapolis, MN 55455
Arch Biochem Biophys; 179(2):495-505 1977

The binding activity obtained from an established line of hepatoma tissue culture (HTC) cells was investigated and found to have a lower affinity for cyclic AMP at physiological pH than the activity from normal rat liver. The apparent binding affinity of HTC preparations could be increased reversibly by adding NaCl or guanidine-HCl. In the presence of these activating substances, a macromolecular inhibitory activity was chromatographically separated from the cyclic MP-binding activity. Removal of this inhibitory component caused the affinity of the cyclic AMP-binding activity from HTC cells to increase and resemble that observed with liver preparations. Adding the isolated inhibitory component back to HTC cells resulted in a decrease in their affinity for cyclic AMP. The inhibitory component is devoid of cyclic AMP-binding and cyclic AMP phosphodiesterase activities, has a molecular wt of 30,000, has protein kinase activity, and seems to be identical to the catalytic subunit of a cyclic AMP-stimulated protein kinase on the basis of chromatographic properties and sensitivities to heat and low pH. This catalytic subunit is a minor portion of total cellular protein kinase activity and is also present in liver extracts. The difference in the inhibitory response between liver and HTC preparations, therefore, appears to reflect differences in the cyclic AMP-binding proteins themselves. (34 Refs)

211. ALTERED REGULATION OF CAMP-DEPENDENT PROTEIN KINASE IN A MOUSE LYMPHOMA CELL LINE.

Lasser M, Daniel V
Dept. Biochemistry, Weizmann Inst. Sci, Rehovot, Israel
Isr J Med Sci; 12(11):1355 1976

The ability of cyclic AMP (cAMP) to inhibit growth, cause cytolysis, and induce synthesis of cAMP-phosphodiesterase in S49 mouse lymphoma cells is lacking in mutant cells selected on the basis of their resistance to killing by 2 mM dibutyryl cAMP (R cells). Compared with the wild-type lymphoma, the resistant cells showed reduced activity of cAMP-binding protein and cAMP-dependent protein kinase activity. The protein kinase activity from R-cell cytosol exhibited an apparent K-a for activation by cAMP that was 100-fold greater than that of the enzyme from the parental cells. The altered protein kinase regulation R cells, which related to the lowered affinity of its regulatory subunit for cAMP, accounts for the insensitivity of these cells to the biologic effects of cAMP. The R-cell cytosol also showed an increase in the activity of a cAMP-independent protein. (no refs)

212. ALTERED CYCLIC AMP-BINDING AND DB CYCLIC AMP-UNRESPONSIVENESS IN VIVO.

Cho-Chung Y, Clair T
Lab. Pathophysiology, NCI, Bethesda, MD 20014
Nature (Lond); 265(5593):452-454 1977

Since defective cyclic AMP-binding proteins may be the possible cause for loss of growth control by cyclic AMP (cAMP), the relationship between the qualitative difference of cAMP-binding proteins and db cAMP-responsiveness was investigated in vivo. Experimental tumor models used included transplantable mammary carcinoma (MTW9) in Wistar-Furth inbred female rats, N-nitrosomethylurea-induced mammary carcinoma and adenocarcinoma (R323OAC) in Fischer female rats, and hepatoma 5123 in Buffalo/N female rats. The high-affinity binding activity of cytosols from unresponsive tumors growing under db cAMP treatment was reduced to n 50% or less when cytosol was pre-incubated, but greater than 75% of binding activity of cytosols from responsive tumors that regressed under db cAMP treatment was retained. Binding site experiments indicate a difference in the structure or conformation of the binding proteins between the responsive and unresponsive tumors. For 44/52 tumors there was a striking parallel between stable cytoplasmic cAMP-binding proteins and db cAMP-induced tumor regression in vivo. The thermal lability of cAMP-binding was associated with a reduced affinity of the binding proteins for cAMP. Nuclear accumulation of cAMP-binding and protein kinase components was not found in the db cAMP-unresponsive tumor following the db cAMP treatment. These studies show a striking parallel between heat stability of cAMP-binding and arrest of growth due to exogenous db cAMP. These findings indicate that unresponsiveness to db cAMP treatment is closely associated with qualitative defects in cAMP binding proteins; thus, the heat stability test of cytosol cAMP-binding proteins could be used as a simple diagnostic method to assess the efficacy of cAMP treatment of tumors. (20 refs)

213. SOLUBILIZED NUCLEAR DNA-DEPENDENT RNA POLYMERASES FROM NORMAL RAT MAMMARY GLANDS AND FROM TRANSPLANTABLE R-35 RAT MAMMARY TUMORS.

Anderson KM, Mendelson IS
Faculty Medicine, Banting Inst., Univ. Toronto, Toronto, Ontario, Canada
Oncology; 31(5-6):338-356 1975

The distribution of nuclear RNA polymerases solubilized from rat mammary tumors, and some effects of cyclic nucleotides on their apparent activity, are reported. The three major nuclear DNA-dependent RNA polymerases (enzymes I, II and III) were present in nuclear extracts from transplantable R-35 rat mammary tumors. Except for somewhat less enzyme III, their relative distribution resembled that of nuclear extracts from late-pregnant rats. When enzyme II from normal tissue extracts was incubated for RNA synthesis with cyclic AMP, inhibition was frequently observed, but this occurred less often with nuclear extracts from the R-35 tumor. In some experiments with both normal and tumor tissue, cyclic AMP and cyclic guanosine monophosphate (cyclic GMP) increased the apparent activity of nucleolar enzyme Ib and nucleoplasmic enzyme II, respectively. Nuclear extracts from both normal and tumor tissue were found to contain proteins that bind radioactive cyclic AMP and cyclic GMP. Their patterns of binding were not identical. These results are consistent with the following hypothesis: altered binding by the tumor of cyclic nucleotides to putative nuclear 'regulatory' proteins (e.g protein kinase subunits, or possibly other high affinity cyclic nucleotide-binding proteins unrelated to protein kinases) contributes to and may be responsible for some of the differences in response to cyclic nucleotides that were

observed. It is possible that such defects occur in other tumors, or even represent a fundamental defect in all cancer cells.

214. PURIFICATION OF PHOSPHOPROTEIN PHOSPHATASE FROM BOVINE CARDIAC MUSCLE THAT CATALYZES DEPHOSPHORYLATION OF CYCLIC AMP-BINDING PROTEIN COMPONENT OF PROTEIN KINASE.

Chou CK, Alfano J, Rosen OM
Dept of Medicine and Molecular Biology, Albert Einstein Coll of Medicine, Bronx, NY 10461
J Biol Chem; 252(9):2855-2859 1977

A phosphoprotein phosphatase that catalyzes the dephosphorylation of cyclic adenosine 3':5'-monophosphate (cAMP)-dependent protein kinase from bovine cardiac muscle has been purified to homogeneity by a modification of the procedure of Brandt et al. (Brandt, H, Capulong, Z L, and Lee, E Y C (1975) J Biol Chem 250, 8038-8044). Treatment of the enzyme preparation with ethanol during the early stages of purification results in activation concomitant with reduction in molecular weight to 30,000. The purified activated enzyme has a Km for phosphoprotein kinase in the presence or absence of 1.2 mM Mn^{2+} of 5 and 22 mum, respectively. Phosphatase activity on phosphoprotein kinase but not on other phosphoprotein substrates was cAMP-dependent. This selective activation by cAMP reflects the preference of the phosphatase for the free, phosphorylated cAMP-binding protein rather than the phosphoholoenzyme. (36 Refs). (Author Abstract)

215. DISSOCIATION AND REASSOCIATION OF THE PHOSPHORYLATED AND NONPHOSPHORYLATED FORMS OF ADENOSINE 3':5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE FROM BOVINE CARDIAC MUSCLE.

Rangel-Aldao R, Rosen OM
Department of Medicine and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461
J Biol Chem; 251(11):3375-3380 1976

Adenosine 3':5'-monophosphate (cyclic AMP)-dependent protein kinase from bovine heart muscle catalyzes the phosphorylation of its regulatory, cyclic AMP-binding subunit. Phosphorylation enhances net dissociation of the enzyme by cyclic AMP. Chromatography on omega-aminohexyl-agarose was used to study the effects of phosphorylation on cyclic AMP binding and subunit dissociation and reassociation. This method permitted rapid separation of the catalytic subunit from the cyclic AMP-binding protein and holoenzyme. Phospho- and dephosphoprotein kinases were found to dissociate to the same extent at any given concentration of cyclic AMP and completely at saturation. At equilibrium, the amount of cyclic AMP bound was the same for both forms of enzyme and was directly proportional to the degree of dissociation of the holoenzyme. In the absence of cyclic AMP, phospho- and dephospho-cyclic AMP-binding proteins reassociated completely with the catalytic subunit. However, the rate of reassociation of the dephospho-cyclic AMP-binding protein was at least 5 times greater than the phospho-cyclic AMP-binding protein. Retardation of reassociation was directly proportional to the extent of phosphorylation. We conclude that the degree to which the cyclic AMP-binding protein is phosphorylated markedly affects its intrinsic ability to combine with the catalytic subunit to regenerate the inactive cyclic nucleotide-dependent kinase and that the state of phosphorylation of this subunit may be important in determining the proportion of dissociated (active) and reassociated (inactive) protein kinase at any given time. (Author Abstract)

216. RADIOIMMUNOASSAY OF BOVINE HEART PROTEIN KINASE (MEETING ABSTRACT).

Fleischer N, Rosen OM, Reichlin M
Departments of Medicine and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461; and the Department of Medicine and Biochemistry, State University of New York, Buffalo, N.Y. 14214
Proc Natl Acad Sci USA; 73(1):54-58 1976

Immunization of guinea pigs with bovine cardiac cAMP-dependent protein kinase (ATP:protein phosphotransferase, EC 2.7.1.37) resulted in the development of precipitating antibodies to the cAMP-binding subunit of the enzyme. Both the phosphorylated and nonphosphorylated cAMP-binding protein of the protein kinase reacted with the antiserum. A radioimmunoassay was developed that detects 10 ng of holoenzyme and permits measurement of enzyme concentration in bovine cardiac muscle. Bovine liver, kidney, brain, and skeletal muscle contain protein kinases which, are immunologically identical to those found in bovine cardiac muscle. However, the proportion of immunoreactive enzyme activity differed for each tissue. All of the immunologically nonreactive enzyme is skeletal muscle and heart was separable from immunoreactive enzyme by chromatography on DEAE-cellulose. Rat tissues and pig heart contained protein kinase activity that cross-reacted immunologically in a nonparallel fashion with bovine cardiac enzyme. These results indicate that cAMP-dependent protein kinases within and between species are immunologically heterogeneous.

217. PROPERTIES OF THE CYCLIC AMP-DEPENDENT PROTEIN KINASE FROM BOVINE AND PORCINE HEART.

Rosen OM, Rubin CS, Erlichman J
Albert Einstein Coll. Medicine, Bronx, NY
Adv Enzyme Regul; 13:173-185 1975

The properties of the cyclic AMP-dependent protein kinase from porcine and bovine heart are studied. Phosphorylation modified the properties of cyclic AMP-dependent protein kinase. The biochemical properties of the protein kinase purified from beef heart were very similar to those reported for other tissues; it catalyzed the transfer of phosphate from ATP to serine and threonine residues in lysine-rich histone, arginine-rich histone, and protamine. Beef heart protein kinase was determined to be an asymmetric protein composed of four polypeptide chains, as asymmetric cyclic AMP-binding protein dimer, and two globular catalytic subunits. As in the case of the kinase purified from bovine heart, dissociation of the partially purified swine heart enzyme by cyclic AMP was facilitated greatly by prior autophosphorylation. It is concluded that autophosphorylation and the concomitant or consequent facilitation of cyclic AMP-induced dissociation occur in more than one protein kinase.

218. REVERSIBLE AUTOPHOSPHORYLATION OF A CYCLIC 3':5'-AMP-DEPENDENT PROTEIN KINASE FROM BOVINE CARDIAC MUSCLE.

Rosen OM, Erlichman J
Departments of Medicine and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461
J Biol Chem; 250(19):7788-7794 1975

Purified cyclic adenosine 3':5'-monophosphate (cAMP)-dependent protein kinase of bovine cardiac muscle catalyzes the incorporation of 2 mol of ^{32}P from (gamma-^{32}P)ATP to seryl residues in its cAMP-binding protein. The reaction appears to be catalyzed by the protein kinase itself rather than by a protein kinase kinase and is enhanced by cAMP and by the addition of polyarginine. Phosphorylation of the purified enzyme facilitates its dissociation by cAMP (Erlichman J, Rosenfeld R and Rosen OM, J Biol Chem; 249: but does not

affect cAMP binding. At equilibrium, 2 mol of cAMP are bound to both the phospho- and dephospho-enzymes. Phosphorylation of protein kinase is reversible. Upon addition of ADP and Mg2+, phosphate is transferred from the protein to ADP, and ATP, is formed. The reverse reaction is optimal at pH 5.5 unlike the forward reaction which has a broad, more alkaline pH activity optimum. It is activated by polyarginine and dependent upon the addition of cAMP to a much greater degree than the forward reaction. The data suggest that the catalytic subunit of protein kinase catalyzes the forward and reverse reactions but do not exclude the possibility that the holoenzyme may also be activated by autophosphorylation by protein kinase and dephosphorylation by phosphoprotein phosphatases or by reversal of the autophosphorylation reaction may regulate the sensitivity of certain protein kinases to activation by cAMP in vivo. (Author Abstract)

219. PHOTOAFFINITY LABELING OF A PROTEIN KINASE FROM BOVINE BRAIN WITH 8-AZIDOADENOSINE 3',5'-MONOPHOSPHATE.

Pomerantz AH, Rudolph SA, Haley BE, Greengard P
Yale Univ. Sch. Medicine, New Haven, Conn. 06510
Biochemistry (Washington): 16(17):3858-3862 1975

8-Azidoadenosine 3',5'-monophosphate (8-N3 -cAMP) containing ^{32}P has been used as a photoaffinity label specific for the adenosine 3',5'-monophosphate (cAMP) binding site(s) present in a partially purified preparation of soluble protein kinase from bovine brain. 8-N3 -cAMP and cAMP were found to compete for the same binding site(s) in this preparation, as determined by a standard filter assay. When this protein preparation was equilibrated with ^{32}P-8-N3 -cAMP, and then irradiated at 253.7 nm, the incorporation of radioactivity was predominantly into a protein with an apparent molecular wt of 49,000, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. This labeled protein comigrated in the gel with the only protein which is endogenously phosphorylated by gamma-^{32} PATP, a protein which has been shown to be the regulatory subunit of the protein kinase. The incorporation of ^{32}P-8-N3 -cAMP into this protein was half-maximal at a concentration of 7 x 10**-8 M. In accordance with a proposed mechanism involving the formation of a highly reactive nitrene intermediate upon irradiation of the azide, the incorporation of radioactivity into protein was maximal within 10 min of irradiation, and was almost eliminated by preirradiation of the photolabile ligand. Moreover, this incorporation was virtually abolished by a 50-fold excess of cAMP, but not by AMP, ADP, ATP, or adenosine. It is suggested that 8-N3 -cAMP may prove to be a useful molecular probe of the cAMP-binding site in receptor proteins. Its affinity for the receptor binding site is similar to that of cAMP, its selectivity is high in a heterogeneous protein mixture, and covalent incorporation is rapid upon irradiation. Its use is reported in conjunction with sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a highly sensitive and selective radiochemical marker for cAMP-binding proteins.

220. SELECTIVE EFFECTS OF MGCL2 AND TEMPERATURE ON THE INITIATION OF TRANSCRIPTION AT LAC, GAL, AND L PROMOTERS.

Nakanishi S, Adhya S, Gottesman M, Pastan I
Natl. Cancer Inst., Bethesda, Md. 20014
J Biol Chem: 250(20):8202-8208 1975

The effect of Mg**+2 on the formation of transcription preinitiation complexes (open complexes) at 2 cyclic AMP-cyclic AMP receptor protein (CRP)-dependent promoters (lac and gal) and 2 phage lambda promoters, PL and PR were studied. Mg**+2 strongly interferes with open complex formation at the lac and gal promoters, partially inhibits the lambda PR promoter, and is without effect on the lambda PL promoter. Mutations in the lac and gal promoters can affect the response of the promoter to Mg**+2. Cyclic AMP and CRP specifically reduce the inhibition of the lac and gal promoters by Mg**+2. These factors also affect open complexes at lac and gal by increasing the rate at which they are formed and lowering the midpoint of the temperature transition curve for their formation by about 10 C. Open complexes at the lac promoter are more unstable to cooling, even in the presence of CRP and cyclic AMP, than open complexes at lambda promoters. Results suggest that the DNA of the lac and gal promoter regions is more resistant to denaturation than the DNA of phage promoter regions. Cyclic AMP and CRP act to decrease this stability, stimulating open complex formation under conditions unfavorable for DNA melting (such as low temperature and high Mg**+2 concentrations).

221. ADENOSINE 3':5'-MONOPHOSPHATE-REGULATED PHOSPHORYLATION OF ERYTHROCYTE MEMBRANE PROTEINS SEPARATION OF MEMBRANE-ASSOCIATED CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE FROM ITS ENDOGENOUS SUBSTRATES.

Rubin CS
Departments of Neuroscience and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461
J Biol Chem: 250(23):9044-9052 1975

An adenosine 3':5'-monophosphate (cyclic AMP)-BINDING protein in the human erythrocyte plasma membrane was isotopically labeled using a photoaffinity analog of cyclic AMP, N^6-(ethyl 2-diazomalonyl) cyclic (^{3}H)AMP. The cyclic AMP-binding site is located in a polypeptide chain having a molecular weight of 48,000. Cyclic AMP-binding protein and cyclic AMP-dependent protein kinase were solubilized with 0.5% Triton X 100 in 56 mM sodium borate, pH 8, but ^{32}P-labeled membrane phosphoproteins were retained in the Triton-insoluble fraction, suggesting that the membrane-associated binding protein is not a primary substrate for protein kinase. Triton-solubilized and membrane-associated protein kinase activities were stimulated 15- and 17-fold by cyclic AMP, suggesting that the degree of association between the catalytic and cyclic AMP-binding components was very similar in both preparations. Fractionation and characterization of membrane phosphoproteins have shown that protein III and a co-migrating minor protein are substrates for protein kinase but membrane sialoglycoproteins are not phosphorylated. (Author Abstract)

222. CAMP BINDING, PROTEIN KINASE ACTIVATION AND THE INHIBITION OF CELL GROWTH.

Tisdale MJ, Phillips BJ
Dept. Biochemistry, St. Thomas's Hosp. Medical Sch., London, SE1 7EH, England
Exp Cell Res: 104(1):135-142 1977

The relationship between the effects of adenosine 3',5'-monophosphate (cAMP) and of guanosine 3',5'-monophosphate (cGMP) on cell growth rate, the binding of these nucleotides to specific cytosol receptors, and activation of protein kinase were investigated in four cell lines: TLX5 (transplantable lymphoma), A9H (fibroblastic), and two hybrids formed from the first two lines, A9H/TLXD and A9H/TLXD (tumor). The concentrations for 50% inhibition (ID50) for growth inhibition by dibutyryl cAMP (DB-cAMP) in ug/ml was 26 for TLX5, 270 for A9H, 125 for A9H/TLXD, and 316 for A9H/TLXD (tumor). DB-cGMP had a stimulatory effect on A9H growth at concentrations below 100 ug/ml (max at 10 ug/ml). At 250 ug/ml, DB-cAMP inhibited cell growth by 68%, while at 10, 50, and 250 ug/ml DB-cGMP inhibited cell growth by 70, 69, and 77%, respectively. The

cytosols of the hybrids had significantly greater amounts of binding sites for ^{3}H-cAMP than did either parent. The hybrid of lower malignancy (A9H/TLXD) had the highest binding of cAMP at concentrations above 6 nanoM. The parent cell lines had the highest binding of cGMP. The cellular receptor for cAMP was present in a concentration at least 10-fold that of the receptor for cGMP. The ratio of binding proteins of the cell lines paralleled the intracellular levels of cAMP and the malignancies of these cell lines. Scatchard analysis indicated the presence of two types of binding sites for cAMP: one of high capacity and low affinity and the other of low capacity and high affinity. In all four cell lines, there was only one one type of binding site for cGMP. Except for TLX5, dissociation constants for cGMP binding were approx 10-fold higher than for the highest affinity cAMP binding site. The effect of 100 ug/ml DB-cAMP on the protein kinase activity ratio (-c-AMP/+c-AMP) of A9H cells resulted in maximal stimulation at 5 hr which remained constant thereafter. Thus, almost full activation of the cAMP-dependent protein kinase occurred at a dose of DB-cAMP which caused only 20% inhibition of cell growth. The basal level of kinase activity of A9H/TLXD cells was lowest, while the cAMP-dependent kinase for this cell line showed the greatest stimulation by 2 uM cAMP. With A9H TLXD (tumor), the protein kinase showed only a slightly greater stimulation by cAMP than did the A9H line. The concentration of cAMP needed for half-maximal stimulation of the protein kinase was much less for A9H TLXD than for the other cell lines. Although A9H TLXD (tumor) had a much higher cAMP-binding protein content than A9H and TLX5, only part of this was involved in the activation of a cAMP-dependent protein kinase. (41 refs)

223. A SPECIFIC CYCLIC GUANOSINE 3':5'-MONOPHOSPHATE-BINDING PROTEIN IN CAULOBACTER CRESCENTUS.

Sun IY, Shapiro L, Rosen OM
Department of Molecular Biology, Division of Biological Sciences, and the Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461
J Biol Chem; 250(15):6181-6184 1975

A binding protein specific for cyclic guanosine 3':5'-monophosphate (cyclic GMP) has been partially purified from extracts of the eubacterium Caulobacter crescentus and resolved from cyclic adenosine 3':5'-monophosphate (cyclic AMP)-binding activity. Binding of cyclic GMP is not affected by the addition of cyclic AMP or 5'-GMP, but is inhibited about 50% by a 50-fold molar excess of dibutyryl cyclic GMP or cyclic hypoxanthine 3':5'-monophosphate. The apparent dissociation constant for the cyclic GMP-binding protein complex is 1.1 x 10**-6 M. (Author Abstract)

224. CYCLIC AMP INHIBITABLE PROTEIN KINASE ACTIVITY FROM HELA 65 PLASMA MEMBRANES. (MEETING ABSTRACT).

Lucid SW, Griffin MJ
Oklahoma Medical Res Foundation, Okla City, OK 73104
Fed Proc; 36(3): 689 1977

HeLa 65 cells contain a plasma membrane protein kinase and endogenous substrate. Kinase activity was assayed by phosphorylation of the plasma membrane protein with (gamma-^{32}P) ATP at pH 8.5 (60 mM Tris-HCl), 0.07 mM EDTA. At limiting protein concentrations, the reaction is linear with time for 10 min. The endogenous reaction requires 20 mM Mg**++ and is inhibited by 20 mM Ca**++, NaF or theophylline, but is not inhibited by 20 mM NaCl, 5'-AMP, or Pi. c-AMP at 10**-5 inhibits the reaction. All detergents tested (0.025%) abolish the reaction. Exogenous receptors (histones and casein) are not phosphorylated. The endogenous phosphorylated product is rendered acid soluble by treatment with pronase. The phosphorylated substrate from in vitro and in vivo experiments was unique and of relatively low molecular weight (about 15,000) as shown by analytical SDS-polyacrylamide disc gel electrophoresis. Phosphoserine and possibly phosphothreonine were detected by paper electrophoresis of the acid hydrolysate of radiolabeled membranes from in vitro and in vivo sources. The kinase is present in plasma membranes of M, G1 and S phase cells, but cells pulsed (1 hr) in vivo with ^{32}Pi showed incorporation in plasma membrane protein only during M and S (not in G1). The c-AMP inhibitability of this kinase may have a significant function in regulating membrane function over the cell cycle. (Author Abstract)

225. ELEVATED CYCLIC AMP LEVELS IN HUMAN BREAST-CANCER TISSUE.

Minton JP, Wisenbaugh T, Matthews RH
Dept. Surg., U. Hosp., Ohio St. U., Columbus
J Natl Cancer Inst; 53(1):283-284 1974

Tracer amounts of 3H-cyclic AMP were added to extracts from malignant and benign human breast tumors and normal breast tissue. The samples were subsequently tested for cyclic AMP by radioimmunoassay. The cyclic AMP levels were 15 times higher in the cancer tissues than in the normal breast tissues and 10 times higher than in the benign fibrocystic breast samples; these differences were statistically significant at the 0.01 level. The high levels of cyclic AMP may represent a situation analogous to that in which a deficiency in cyclic AMP binding protein and in cyclic AMP-dependent protein kinase activity makes a population of lymphoma cells resistant to the cytolytic effects of dibutyryl cyclic AMP. In any event, the results obtained should not be interpreted as a negation of the concept that cyclic AMP represses growth and promotes the differentiation of normal tissue.

226. METHODOLOGICAL ASPECTS OF PROTEIN-BINDING ASSAYS FOR CYCLIC AMP IN HUMAN ADIPOSE TISSUE.

Arner P, Ostman J
Huddinge Univ. Hosp., S-141 86 Huddinge, Sweden
Scand J Clin Lab Invest; 35(7):691-697 1975

The specificity of Gilman's protein-binding assay for cyclic AMP was studied in human subcutaneous adipose tissue. A kit assay obtained from Boehringer-Mannheim was used for the study, which involved preincubating adipose tissue for 30 min in Krebs-Heinsleitbicarbonate buffer with theophylline (10**-2 M/liter), followed by a 2-minute incubation in the same medium in the presence or absence of l-isopropylnoradrenaline bitartrate (6 x 10**-5 M/l). After incubation, the tissue was homogenized, extracted, and assayed for intracellular cyclic AMP by the phosphodiesterase test. Impurities in the solvents used for homogenization and extraction led to serious overestimations of the actual amount of cyclic AMP. This was corrected for by treating the standard and tissue samples in the same manner. Extracts from the adipose tissue contained a material that increased the binding of cyclic AMP to the binding protein and led to the underestimation of intracellular cyclic AMP by 60-70%. This was overcome by using protein kinase inhibitor (20 microg) in the assay. Incubation of human adipose tissue in bovine serum albumin resulted in a 30% underestimation of intracellular cyclic AMP since the albumin increased cyclic AMP binding to the binding protein.

B. Relationships of Cyclic Nucleotide Binding Proteins to Differentiated Functions Expressed by Cells In Vitro

227. CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN KINASE ACTIVITY IN MALIGNANT AND CYCLIC AMP-INDUCED "DIFFERENTIATED" NEUROBLASTOMA CELLS IN CULTURE.

Prasad KN, Fogleman D, Gaschler M, Sinha PK, Brown JL
Univ. Colorado Medical Center, Denver, CO 80220
Biochem Biophys Res Commun; 68(4):1248-1255 1976

The activity of adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase was demonstrated in the supernatant (S) fraction (100,000 x g) of mouse and human neuroblastoma (NB) cells. In cAMP-induced "differentiated" mouse NB cells, the cAMP-dependent protein kinase (cAMP-PK) activity did not significantly change. Guanine 3',5'-cyclic monophosphate (cGMP) did not stimulate the PK activity in S-fraction the cAMP-PK or cGMP-PK activity was not detected in the membrane (M) fraction. The levels of binding proteins markedly increased in S-fraction of "differentiated" NB cell, but cAMP-PK activity did not change cAMP binding proteins were present in the M-fraction, but cAMP-PK activity was not demonstrable. Cyclic GMP binds with the soluble proteins with about ten-fold less binding affinity than cAMP; however, cGMP does not stimulate PK activity. The present results, in combination with previous data, support the concept that the major portion of binding proteins is distinct from the regulatory subunits of cAMP-PK. The levels of these binding proteins may be differently regulated.

228. BINDING OF CYCLIC NUCLEOTIDES WITH PROTEINS IN MALIGNANT AND ADENOSINE CYCLIC 3':5'-MONOPHOSPHATE-INDUCED "DIFFERENTIATED" NEUROBLASTOMA CELLS IN CULTURE.

Prasad KN, Sinha PK, Sahu SK, Brown JL
Department of Radiology and Biochemistry, University of Colorado Medical Center, Denver, Colorado 80220
Cancer Res; 36(7):2290-2296 1976

The binding of adenosine cyclic 3':5'-monophosphate (cyclic AMP) with soluble (100,000 x g supernatant), pellet, and total homogenate proteins from cyclic AMP-induced "differentiated" mouse neuroblastoma cells increased by about two-fold. The extent of binding with soluble proteins was higher than that with pellet proteins. The binding of cyclic AMP with soluble proteins from 5'-adenosine monophosphate -treated, serum-free, medium-treated sodium butyrate -treated, 6-thioguanine -treated, or X-irradiated neuroblastoma cells did not significantly change. When the soluble proteins containing bound cyclic (^{3}H)AMP were filtered through a Sephadex G-25 column, the relative amount of protein-bound cyclic (^{3}H)AMP in differentiated cells was greater than that in malignant cells, but the amount of free cyclic (^{3}H)AMP was correspondingly less. The electrophoretic characteristics of cyclic AMP-binding proteins of differentiated and malignant cells were identical. There were two binding peaks, but the extent of binding at each peak was relatively high in differentiated neuroblastoma cells. An increase in cyclic AMP binding occurred 24 hr after treatment of neuroblastoma cells with prostaglandin E . This increase was completely blocked by cycloheximide but not by actinomycin D . The binding was heat labile and sensitive to protease action. These data indicate that the increase in binding in differentiated cells is due to an elevation in the levels of binding proteins. The binding of cyclic AMP with soluble proteins from rat glial cells and mouse L-cells did not significantly change after treatment with prostaglandin E1 or an inhibitor of cyclic AMP phosphodiesterase . Cyclic AMP and guanosine cyclic 3':5'-monophosphate bind with the same proteins, but cyclic AMP has about 10-fold higher binding affinity than does guanosine cyclic 3':5'-monophosphate. (Author Abstract)

229. REGULATION OF CYCLIC AMP BINDING PROTEINS AND CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY IN MOUSE NEUROBLASTOMA CELLS IN CULTURE (MEETING ABSTRACT).

Prasad KN, Sinha PK
Dept. of Radiology, University of Colorado Medical Center, Denver, CO 80220
Fed Proc; 35(3):296 1976

The change in the level of cAMP binding proteins and cAMP-dependent protein kinase (cAMP-PK) activity was investigated during differentiation of mouse neuroblastoma (NB) cells in culture. The level of cAMP binding proteins in the supernatant (S) fraction of cAMP-induced "differentiated" NB cells increases by about 2-fold, but cAMP-PK activity does not change. CAMP binding proteins are present in the membrane (M) fraction but cAMP-PK activity is not detectable cGMP binds with soluble proteins with 10-fold less binding affinity than cAMP; however, cGMP does not stimulate PK activity. An elevation of cAMP in NB cells increases the level of cAMP binding proteins without changing cAMP-PK activity. These data suggest that the major portion of cAMP binding proteins in NB cells is distinct from the regulatory subunits of cAMP-PK, and they are regulated differently. The cAMP binding proteins protect the formed cAMP from enzymatic hydrolysis during differentiation, and thus the increase in binding proteins level provides one of the important intracellular mechanisms of maintaining high cAMP level in NB cells. (Author Abstract)

230. REGULATION OF CYCLIC NUCLEOTIDES IN NEUROBLASTOMA CELLS IN CULTURE (MEETING ABSTRACT).

Prasad KN, Sinha PK
Dept. Radiology, Univ. Colorado Medical Center, Denver, CO 80220
J Cell Biol; 70(2/Part2):325a 1976

The levels of cyclic nucleotides in mouse neuroblastoma cells are dependent not only on the relative activity of cyclases and phosphodiesterases (PDE), but also on the level of cyclic nucleotide binding proteins. Dopamine (DA), norepinephrine (NE) and acetylcholine (ACh) stimulate adenylate cyclase (AC) activity in vitro in some clones, whereas prostagladin E1 (PGE1) stimulates AC activity in all clones. However, DA and NE do not increase the intracellular level of adenosine 3'5'-cyclic monophosphate (cAMP) until PDE activity is inhibited. ACh under a similar experimental condition decrease the cAMP level, but ACh by itself increases the 3',5'-cyclic monophosphate (cGMP) level. PGE1 increases the cAMP level in most clones, but in at least one clone, PGE1 does not increase the cAMP level until the PDE activity is inhibited. Thus the activity of PDE in malignant cells is a limiting factor in the accumulation of cAMP following stimulation by DA, NE, and PGE1 . However, during cAMP-induced differentiation the level of cyclic cAMP binding proteins increases by 2-fold, and thus provides one of the important intracellular mechanisms of maintaining high cAMP level. This may in part explain the fact that the levels of cAMP as well as activity of cAMP PDE increase by 3-4 fold in "differentiated" cells, and the effect of cAMP on several clones is irreversible after the removal of drug. The cAMP-effects on nonneural tumor cells are reversible, because these cells do not increase the level of binding proteins. Although cGMP PDE activity is only half of cAMP PDE, the level of cGMP is 1/100th of the cAMP level. This may in part be due to the fact that the binding affinity of cGMP with protein is 10

times less than cAMP. Thus the relative significance of cyclases, PDE and binding proteins in maintaining the level of cyclic nucleotides may change during differentiation and malignant transformation of nerve cells. (No refs)

231. CYCLIC NUCLEOTIDES IN THE REGULATION OF EXPRESSION OF DIFFERENTIATED FUNCTIONS IN NEUROBLASTOMA CELLS.

Prasad KN, Sahu SK, Sinha PK
Dept. Radiology, Univ. Colorado Medical Center, 4200 E. 9th Ave., Denver, CO 80220
J Natl Cancer Inst; 57(3):619-631 1976

Investigations were conducted of cyclic nucleotides and differentiation, regulation of adenylate cyclase activity, cyclic nucleotides phosphodiesterase activity, cyclic nucleotide-binding proteins, and mechanisms of cyclic AMP effects in neuroblastoma cells cAMP may be important in regulating expression of many differentiated functions in these cells, although some of these functions can be induced by agents that do not increase intracellular cAMP. Neuroblastoma cells showed increased levels of cAMP-binding proteins during differentiation, which might have accounted in part for an increase in intracellular cAMP even in the presence of high phosphodiesterase activity cAMP induced irreversible differentiation by increasing the expression of some genetic information while suppressing expression of others, eg, activity of neural enzymes increased while synthesis of histone and the phosphorylation of H1-histone markedly decreased in differentiated cells. Based upon these results, a hypothesis of malignancy of nerve cells and a new therapeutic approach for treatment of neuroblastoma were developed. An increase in cAMP phosphodiesterase activity as a result of mutation in the regulatory gene for phosphodiesterase in a single, or group of, dividing nerve cell(s) is hypothesized to be the primary lesion leading to malignancy. Suggested modifications of the treatment of human neoplasm included the administration of drugs minimizing tumor burden and retaining the immunologic integrity of the host and administration of agents that would increase cAMP levels and cause differentiation. Differentiated cells would then elicit a cytotoxic host response directed against residual malignant cells. Specifically, treatment would involve administration of sodium butyrate followed by L-dihydroxyphenylalanine and prostaglandin E1 in the presence of cAMP phosphodiesterase inhibitor, followed by the less immunosuppressive vincristine (VCR) and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide. Additional evidence in support of this type of treatment includes results of clinical trials with papaverine used with cyclophosphamide, VCR, and trifluoro-methyl-2-deoxyuridine, in which neuroblastomas have been converted to ganglioneuromas. (56 refs)

232. CYCLIC AMP BINDING PROTEINS: THEIR POSSIBLE SIGNIFICANCE IN THE MALIGNANCY OF NEURAL AND NONNEURAL TUMOR CELLS.

Prasad KN, Sinha PK
Dept. of Radiology, Univ. of Colo. Med. Center, Denver, Co 80220
Proc Am Assoc Cancer Res; 17:156 1976

This study was performed to identify the lesion(s) in cAMP-system of neural and nonneural tumor cells in culture. The level of cAMP binding proteins (cAMP-BP) in the supernatant fraction of cAMP-induced-"differentiated" mouse neuroblastoma (NB) cells increases by about 2-fold, but cAMP-dependent protein kinase (cAMP-PK) activity is unchanged. The membrane fraction contains cAMP-BP without any cAMP-PK activity. Cyclic GMP binds with soluble proteins without stimulating PK activity. These data suggest that the major portion of cAMP-BP in NB cells is distinct from the regulatory subunits of cAMP-PK, and may protect formed cAMP from enzymatic hydrolysis. The irreversible effect of cAMP on NB cells may be associated with the increase in the cAMP-BP level. The cAMP-BP level in rat glial and mouse L-cells after treatment with cAMP stimulating agents does not change. This may in part account for the reversibility of cAMP-effects on nonneural tumor cells. We suggest that a decrease in the cAMP-BP level may be one of the critical lesions of malignancy of some nonneural cells. This defect is not apparent in at least some clones of NB cells in which the primary lesion of malignancy appears to be the increase in cAMP phosphodiesterase activity. (Author Abstract)

233. ABNORMAL REGULATION OF CYCLIC AMP BINDING PROTEINS. A CRITICAL LESION OF MALIGNANCY OF NON-NEURAL CELLS.

Prasad KN, Sinha PK
Univ. Colorado Medical Center, 4200 E. 9th Ave., Denver, CO 80220
Differentiation; 6(1):59-60 1976

On the basis of several reports, it is proposed that a defect in the regulation of cyclic AMP (cAMP) binding proteins may be one of the critical lesions in the cancer cells derived from glial cells and fibroblasts. Mutation in the regulatory gene for cAMP binding proteins in nonneural cells may occur as a result of viruses, chemical carcinogens, ionizing radiation, or any combination of these agents.

234. CYCLIC NUCLEOTIDES AND INTRACELLULAR MEMBRANES IN NORMAL AND NEOPLASTIC LIVER (MEETING ABSTRACT).

Pitot HC, Sharma RK, McLaughlin CA
McArdle Laboratory, University of Wisconsin, Madison, Wisconsin
Proc Am Assoc Cancer Res; 17:120 1976

Studies from this laboratory have demonstrated that the smooth and rough endoplasmic reticulum of normal and neoplastic rat liver possess one and two specific binding sites for cyclic AMP respectively. The constants for cyclic AMP binding to the intracellular membranes of the Morris hepatomas 5123C and 7777 exhibit two binding sites, the binding constant of one comparable to that of cyclic AMP for normal liver membranes whereas the value of the second intrinsic association constant differs by up to 40-fold from that of liver. Reticular membranes of these hepatomas also exhibit a binding site for cyclic GMP which is absent from the intracellular membranes of liver. The intracellular membranes of liver and the Morris 5123C hepatoma also possess endogenous cyclic nucleotide independent protein kinase activity which phosphorylates five groups of proteins in liver but only three of the 5 protein bands in the hepatoma. These findings demonstrate distinct differences in the cyclic nucleotide binding proteins of liver and 2 hepatomas as well as an inability of the cyclic nucleotide independent protein kinase of hepatoma intracellular membranes to phosphorylate protein species that are found in membranes of both liver and the 5123C hepatoma. (Author Abstract)

235. ALTERATIONS IN ADENOSINE 3',5'-MONOPHOSPHATE-BINDING PROTEIN IN WALKER CARCINOMA CELLS SENSITIVE OR RESISTANT TO ALKYLATING AGENTS.

Tisdale MJ, Phillips BJ
Department of Biochemistry, St Thomas' Hospital Medical School, London SE1 7TH England
Biochem Pharmacol; 25(16):1831-1836 1976

The extent of binding of 8-(^{3}H)adenosine 3',5'-monophosphate (cyclic AMP) to specific sites was measured in Walker carcinoma cells of tissue culture lines, having different

degrees of resistance to the cytotoxic effects of alkylating agents. When compared with sensitive cells, the resistant lines showed a loss of binding activity with increasing resistance, when measured at either pH 4.0 or pH 6.5. This applied to cell lines made resistant either to 5-aziridinyl-2,4-dinitrobenzamide (CB 1954) or chlorambucil , although the total loss of binding activity was greater in the former cell lines. Scatchard analysis of binding suggested the presence of two sites in all cell lines with KD1 approximately equal to 1-5 x 10**-9M and KDII approximately equal to 3 x 10**-8M. The decreased binding activity was not due to an increased cAMP phosphodiesterase activity in the resistant cell lines, since the activity of the high affinity form of this enzyme was either the same as (CB 1954-resistant) or less than (chlorambucil-resistant) that found in sensitive cells. Walker cells with acquired resistance to either CB 1954 or chlorambucil showed a degree of cross resistance to the cytostatic effect of cAMP analogues and of other alkylating agents. (Author Abstract)

236. CYCLIC AMP, MEMBRANE TRANSPORT AND CELL DIVISION. I. EFFECTS OF VARIOUS CHEMICALS ON CYCLIC AMP LEVELS AND RATE OF TRANSPORT OF NUCLEOSIDES, HYPOXANTHINE AND DEOXYGLUCOSE IN SEVERAL LINES OF CULTURED CELLS.

Sheppard JR, Plagemann PG
Dight Inst. Hum. Genet., Univ. Minnesota, Minneapolis
J Cell Physiol; 85(2):163-172 1975

The relationship between the cellular cyclic AMP level and uridine , thymidine , hypoxanthine , and deoxyglucose transport was studied in Novikoff rat hepatoma cells, Polyoma virus-transformed 3T3 cells, 3T6, C6 glioma cells, and mouse L cells. Cell suspensions or plate cultures were incubated with papaverine , prostaglandin E1 , isoproterenol , Persantin , Cytochalasin B , or dibutyryl cyclic AMP and 3H-labeled substrate. Papaverine, prostaglandin E1, and isoproterenol-induced changes in the intracellular cyclic AMP levels within the test cultures were not correlated with the inhibition of uridine, hypoxanthine, or deoxyglucose transport. Chemically-induced inhibitions in the transport rates occurred in most cell lines in the absence of any measurable change in the intracellular cyclic AMP concentration. Furthermore, treatment of several cell lines with 1 mM dibutyryl cyclic AMP had no immediate effect on the transport of uridine, thymidine, or deoxyglucose, although the transport capacity of the cells for uridine and thymidine, but not that for deoxyglucose, decreased progressively with time. Although the uridine transport system of the 3T3-derived cells and the hypoxanthine transport system of the L cells exhibited high degrees of resistance to inhibition by the various chemicals tested, deoxyglucose transport was inhibited to about the same extent in all cell lines. The results suggest that the competitive inhibitions may be due to the binding of the inhibitor to a binding site different from the substrate binding site, but located in close proximity to the latter. Alternatively, it is possible that the inhibitors affect transport processes by competitively binding to the substrate binding sites and that the binding proteins of different cell lines exhibit different affinities for the inhibitors. The data do not support the hypothesis that cellular cyclic AMP levels and cellular transport systems are related and that they cooperatively control cell division.

237. DIFFERENCES BETWEEN LIVER AND HEPATOMA CELLS IN THEIR COMPLEMENTS OF ADENOSINE 3':5'-MONOPHOSPHATE-BINDING PROTEINS AND PROTEIN KINASES.

Mackenzie III CW, Stellwagen RH
Univ. Southern California Sch. Medicine, Los Angeles
J Biol Chem; 249(18):5755-5762 1974

The differences between liver cells (from male Buffalo rats) and hepatoma tissue culture cells (HTC) in both cyclic AMP-binding proteins and protein kinases were compared to investigate the mechanism by which cyclic AMP induces enzymes in eukaryotes. Scatchard plots for the binding of cyclic AMP were compared at pH 4 and 6.5. At both pH values, liver showed nonlinear plots which were increased in curvature and apparent affinities by prior dialysis of the samples, mostly due to removal of endogenous cyclic AMP. For HTC cells the Scatchard plot at pH 4 was also nonlinear and exhibited high binding affinity (KDx0.5 nM), but prior dialysis was unnecessary and the shape of the curve differed from that of liver. At pH 6.5, the binding affinity observed in HTC extracts was lower than seen with liver and was insensitive to dialysis for 1-2 days. However, this binding could be partially converted to higher affinity by aging or by overnight treatment at pH 4. The binding activity present in liver extracts was considerably less stable than that from HTC cells at pH 4 in the absence of cyclic AMP. Increasing the pH and adding cyclic AMP stabilized the binding activities from both sources. Much of the nonlinearity observed in the Scatchard binding plots was in the opposite direction from that caused by the instability of binding components at low concentrations of cyclic AMP. Chromatography on DEAE-cellulose showed that HTC extracts lacked one of two major cyclic AMP-stimulated protein kinase fractions which were present in liver. The deficiency was apparently due primarily to the absence of a cyclic AMP-binding fraction. Chromatographically analogous protein kinase peaks were present in both HTC and liver extracts, although their proportions differed. The cyclic AMP-binding fraction in liver was very labile at pH 4 and accounted for the greater instability of binding activity in liver extracts compared with HTC samples. HTC cells and liver also differed in the properties of the cyclic AMP-stimulated protein kinase fraction common to both. The activity of this chromatographic fraction from HTC cells was less responsive to cyclic AMP and had a lower binding affinity than that from liver. This lower HTC binding affinity could be increased by the same treatments which were effective with unfractionated extracts.

238. HETEROGENEITY AND UNUSUALLY HIGH AFFINITY IN THE INTERACTIONS OF ADENOSINE 3':5'-MONOPHOSPHATE WITH SPECIFIC BINDING PROTEINS FROM LIVER AND HEPATOMA CELLS.

Mackenzie III CW, Stellwagen RH
Univ. Southern California Sch. Medicine, Los Angeles
J Biol Chem; 249(18):5763-5771 1974

The binding of cyclic AMP to proteins was investigated in post-mitochondrial supernatant fractions from rat liver and cultured hepatoma (HTC) cells. Examination of the membrane filtration method used to measure the binding showed that essentially all of the protein in the extracts is retained and that nonspecific adsorption of cyclic AMP is not a serious problem. At high concentrations of cyclic AMP, the amount of binding increased linearly over a range of protein concentrations. The binding was highly selective for cyclic AMP as shown by competition studies with related compounds. The time required to reach binding equilibrium at 0 C varied with the pH, the nature of the sample, and the cyclic AMP concentration. The kinetics of release of bound cyclic AMP were heterogeneous for both liver and HTC extracts,

showing a range of half times from minutes to days. An evaluation of the instability of binding activity under assay conditions showed that this instability could not cause the upward curvature seen in Scatchard plots, but it could account for the additional plateau or region of downward curvature observed with liver samples. Prior exposure of a liver sample to pH 4 or to elevated temperatures eliminated the component(s) responsible for the plateau and transformed the Scatchard binding curve at pH 4 into one showing only the upward curvature characteristic of HTC samples. The high affinity portion of the heterogeneous Scatchard plots was markedly reduced by increasing the salt concentration in the binding reaction at pH 4, but not at pH 6.5. Treatment with HgCl2 in the cold also produced some selective elimination of the higher affinity portion of the HTC Scatchard binding curve. Although these selective effects on different regions of the HTC Scatchard plot were consistent with the presence of multiple components with difiering affinities for cyclic AMP, other explanations could not be ruled out. Negatively cooperative interactions among identical binding sites would also account for the curvature, and these interactions might be sensitive to some of the agents used. In addition, theoretical considerations showed that the interaction between a single binding component and the catalytic subunit of a protein kinase could generate nonlinear Scatchard plots similar to those observed.

239. NUCLEAR CYCLIC NUCLEOTIDE BINDING PROTEINS, RNA SYNTHESIS AND CANCER.

Anderson KM
Dept. Clinical Biochemistry, Faculty Medicine, Univ. Toronto, 100 College St., Toronto, Ontario, M5G 1L5, Canada
Differentiation; 4(3):197-200 1975

The relationships between nuclear cyclic nucleotide binding proteins, RNA synthesis and cancer are discussed. The topics discussed include the following: increasing evidence for effects of cyclic nucleotides on nuclear RNA synthesis; the presence of cyclic nucleotide dependent and independent protein kinases and of binding proteins with a high affinity for cyclic AMP and cyclic guanidine monophosphate (GMP) in cytosol preparations from various tissues, including rat mammary gland nuclear extracts; the fact that phosphorylation increases the ability of RNA polymerases I and II to transcribe DNA; the fact that protein kinases sensitive to cyclic AMP are deficient or absent in the cytosol of several Morris hepatomas and that the distribution of cytosol cyclic AMP and cyclic GMP binding proteins differs from normal rat liver; and the presence of abnormal regulatory molecules which are responsible for RNA synthesis. (61 refs)

III. OTHER CELL SURFACE RECEPTORS

A. Reactions of Lectins and Mitogens with Cell Surface Receptors

240. CHARACTERIZATION OF THE LYMPHOCYTE SURFACE RECEPTORS FOR CON A AND PHA.

Henkart PA, Fisher RI
Natl. Cancer Inst., Bethesda, Md.
J Immunol; 114(2):710-714 1975

Human peripheral blood lymphocytes from normal donors were iodinated with 131I by the lactoperoxidase method to study the molecular nature of the cell surface receptors for concanavalin A (Con A) and phytohemagglutinin (PHA). Following iodination, living lymphocytes were separated by density gradient centrifugation, and their membranes were solubilized. Aliquots of the membrane were incubated with Con A and PHA mitogens and antimitogen antibody. The precipitates formed were subjected to gel electrophoresis for analysis of protein binding by the mitogens. The patterns of protein binding of Con A and PHA were quite similar, but Con A bound two times the amount of labelled protein as PHA did. The binding patterns showed multiple peaks indicating that many surface proteins, ranging in molecular weight from 43,000 to 68,000 daltons, bound these mitogens. The patterns obtained from the freshly drawn lymphocytes were comparable to those obtained from long-term lymphoid tissue culture cell lines. The results indicate that the heterogeneity of the receptors binding Con A and PHA is not due exclusively to lymphocyte heterogeneity, and that no specific cell surface receptor is responsible for the binding of these mitogens.

241. MOBILITY OF CARBOHYDRATE-CONTAINING STRUCTURES ON THE SURFACE MEMBRANE AND THE NORMAL DIFFERENTIATION OF MYELOID LEUKEMIC CELLS TO MACROPHAGES AND GRANULOCYTES (MEETING ABSTRACT).

Inbar M, Ben-Bassat H, Fibach E, Sachs L
Dept. Genet, Weizmann Inst. Sci., Rehovot, Israel
Proc Natl Acad Sci USA; 70(9):2577-2581 1973

Clones (D+) of a cultured line of myeloid leukemic cells from SL mice can be induced to undergo normal differentiation to mature macrophages and granulocytes. Other clones (D-) derived from the same cell line could not be induced to differentiate. The carbohydrate-binding protein concanavalin A (Con A) was used as a probe to study the mobility of carbohydrate-containing sites on the surface membrane of these cells. Changes in the distribution of Con A binding sites on the surface membrane can be induced by Con A. With the appropriate site mobility, this induction of a new distribution resulted in a concentration of Con A-membrane site complexes on one pole of the cell to form a cap. D+ and D- clones showed 50 and 5% of cells with caps, resp, although both types of cells bound a similar number of Con A molecules. Treatment of cells with trypsin increased cap formation from 5 to 40% in D- cells, but did not change the percentage of cells with caps in D+ cells. The results show a difference in the mobility of Con A binding sites in these two types of cells and suggest a difference in the fluid state of these carbohydrate-containing structures on the surface membrane. It is suggested that a gain of the ability of myeloid leukemic cells to undergo normal differentiation is associated with an increase in the fluidity of structures on the surface membrane where the Con A sites are located. Differences in fluidity of specific membrane sites may also explain differences in the response of cells to other differentiation-inducing stimuli.

242. REARRANGEMENT OF CONCANAVALIN A RECEPTOR SITES ON CELLS TAGGED WITH DINITROFLUOROBENZENE (DNFB): EVIDENCE FOR THE CHEMICAL INDUCTION OF A CHANGE USUALLY ASSOCIATED WITH MALIGNANT TRANSFORMATION (MEETING ABSTRACT).

Madyastha KR, Barth RF, Madyastha PR
Dept of Pathology and Oncology, Univ of Kansas Medical Center, Kansas City, Ks 66103
Proc Am Assoc Cancer Res; 17:126 1976

Normal human peripheral blood granulocytes and lymphocytes which are tagged with DNFB are agglutinated by either Con A or Wheat Germ Agglutinin in a way which resembles leukemic cells. The present study further defines this reaction. The binding of Con A to untagged and DNP-tagged granulocytes, treated with DNFB at a ratio of 10^4 molecules per cell, was quantified by isotopic dilution experiments employing ^{3}H-Con A. Similar amounts of the lectin were bound to untagged and DNP-tagged cells following incubation for 5 minutes at 0 degrees or 30 minutes at 24

degrees C: 1.1 x 10^5 molecules/cell, 4.6 x 10^2/mu^2 of surface area, and 1.6 x 10^3/microgm of protein. The role of microtubules in the agglutination of DNP-tagged cells was studied by adding either Vinblastine or Colchicine to the reaction mixture. This produced a quantitative decrease in agglutination which could be attributed to a block during the second stage of the reaction. Aldehyde fixation, which immobilizes Con A receptor sites, completely abolished agglutination although similar amounts of the lectin were bound by both unfixed and aldehyde-fixed cells. We conclude that dinitrophenylation of normal cells may produce a rearrangement of Con A receptors which resembles the clustering of sites usually found on malignant cells. (Author Abstract)

243. FLUIDITY OF MEMBRANE LIPIDS AND LATERAL MOBILITY OF CONCANAVALIN A RECEPTORS IN THE CELL SURFACE OF NORMAL LYMPHOCYTES AND LYMPHOCYTES FROM PATIENTS WITH MALIGNANT LYMPHOMAS AND LEUKEMIAS.

Ben-Bassat H, Polliak A, Rosenbaum SM, Naparstek E, Shouval D, Inbar M
Chanock Center for Virology, Dept of Hematology and Medicine A, The Hebrew University-Hadassah Medical School, Jerusalem
Cancer Res; 37(5):1307-1312 1977

Lymphocytes isolated from the peripheral blood of patients with nonmalignant and malignant disorders were studied for fluidity of membrane lipids and lateral mobility of concanavalin A (Con A) receptors. The degree of fluidity of the surface membrane lipid core was monitored quantitatively by fluorescence polarization analysis using the probe 1,6-diphenyl-1,3,5-hexatriene embedded in lipid regions of the surface membrane of intact cells. Mobility of Con A surface receptors was determined by the cap-forming ability after binding of fluorescent Con A. The present studies were performed on lymphocytes from 28 patients with malignant lymphomas, 22 patients with leukemia, 28 individuals who either were healthy or had nonmalignant disorders, and 5 patients with carcinoma. The results showed that lymphocytes and mononuclear cells from patients with malignant lymphomas and leukemias have a more fluid lipid layer in their surface membrane than do lymphocytes obtained from healthy individuals or from patients with other malignant and nonmalignant disorders. This increase in membrane fluidity was less pronounced in lymphocytes isolated from leukemic patients in clinical remission and from leukemic patients receiving treatment with steroids. The results also show a marked difference in the cap-forming ability of lymphocytes from patients with malignant lymphomas or leukemia as compared with lymphocytes from patients with nonmalignant disorders or carcinoma. Lymphocytes isolated from lymphoma and chronic lymphatic leukemia patients during remission stages of the disease exhibited a higher cap-forming ability. The cap-forming ability of cells from patients with chronic lymphocytic leukemia was unaffected by treatment with steroids. The present results, which are in line with previous observations, have shown that normal lymphocytes can be characterized by a low degree of lipid fluidity but a high degree of mobility of Con A receptors, whereas leukemic lymphocytes are characterized by a high degree of lipid fluidity but a low degree of mobility of Con A receptors. These results confirmed our general hypothesis on the dynamic interrelation between membrane lipids and membrane protein receptors, and they indicate that the widely accepted term "membrane fluidity" requires better consideration for different membrane components. (28 Refs) (Author Abstract)

244. RECEPTOR MOBILITY AND RECEPTOR CYTOPLASMIC INTERACTIONS IN LYMPHOCYTES (MEETING ABSTRACT).

Edelman GM, Yahara I, Wang JL
Rockefeller U., New York, N.Y.
Proc Natl Acad Sci USA; 70(5):1442-1446 1973

Experimental findings from the literature have been correlated to formulate a hypothesis concerning membrane receptor-cytoplasmic interactions. Based primarily on studies of the inhibition by concanavalin A (conA) of lymphocyte surface receptor mobility, it has been proposed that binding of multivalent lectins alters the interaction of an assembly of colchicine-binding proteins with lectin receptors on the surface and other receptors, and reciprocally that the state of the colchicine-binding assembly alters the mobility and distribution of surface receptors on the cell membrane. Support for this hypothesis has come from experiments in which colchicine, colcemid, vinblastine and vincristine were found to reverse partially the effect of con A on receptor mobility and in which colchicine and vinblastine inhibited the agglutination of fibroblasts with neutrophils by con A. The hypothesis requires that some of the cell surface receptors are anchored on, in, or under the lymphocyte plasma membrane. The bulk of evidence favors a cytoplasmic origin for these receptors which may be related to actin-like proteins or microtubules. Constantly changing mobility of these receptors would provide a mechanism whereby external stimuli may be transmitted to the interior of the cell to elicit such responses as contact inhibition, mitosis or cellular motility.

245. LIGAND-INDUCED REDISTRIBUTION OF CONCANAVALIN A RECEPTORS ON NORMAL, TRYPSINIZED AND TRANSFORMED FIBROBLASTS.

De Petris S, Raff MC, Mallucci L
Zool. Dept., U. Coll. London, England
Nature New Biol; 245(139):275-278 1973

It was suggested in previous work that the increased concanavalin A (con-A) agglutination of transformed cells compared to normal cells results from differences in the arrangements of cell surface lectin receptors in the different types of cells. Labelling of receptors with con A-ferritin (con A-FT) or with con A-peroxidase had indicated random distribution of receptors in normal cells, clustered distribution in transformed cells. To determine whether the clustered distribution in transformed cells may have been secondarily induced by the binding of the multivalent con A, attempts were made to correlate the pattern of induced redistribution and agglutinability in normal, trypsinized and transformed cells, using thin section electron microscopy of con A-FT-treated cells. The cell systems tested were normal mouse 3T3 cells, 3T3 cells transformed by polyoma virus (PV-3T3), BALB/c secondary mouse embryo fibroblasts (MEF), C57BL/6 tertiary mouse embryo fibroblasts, and C57BL/6 fibroblasts transformed by polyoma virus (PV-TT3). In certain experiments cells prefixed with glutaraldehyde were used. This was expected to immobilize surface proteins without substantially altering the ability of the carbohydrate moiety of glycoproteins to bind con A. Normal 3T3 cells were only slightly agglutinated by con A, while transformed PV-3T3 and, even more markedly, trypsinized 3T3 cells were strongly agglutinated. Normal MEF were also agglutinated, although agglutination was enhanced after trypsinization. When prefixed cells were labelled with con A-FT, the FT molecules appeared to cover all parts of the membrane, including microvilli, without appreciable differences between normal, trypsinized

or transformed cells. When cells were incubated with con A-FT before fixation, the distribution was no longer uniform and ferritin was irregularly concentrated in patches of variable size. Further, the amount of labelling and the degree of patching were not appreciably different in normal, trypsinized or transformed cells, in spite of their marked differences in agglutinability. It was concluded that the patching of con A receptors is a secondary phenomenon, probably induced by cross linking by the multivalent con A. This was further supported by the enhancement of patching by anti-FT antibodies. The results suggest that clustering of receptors, although perhaps important and even necessary for agglutination, is not in itself sufficient to explain differences in agglutination, at least when induced by con A-FT. Morphological characteristics of the cell surface, such as number and distribution of microvilli, could influence agglutination, but cannot explain the difference in agglutination between normal and trypsinized cells where these features were similar.

246. REQUIREMENT FOR CELL REPLICATION AFTER SV40 INFECTION FOR A STRUCTURAL CHANGE OF THE CELL SURFACE MEMBRANE.

Ben-Bassat H, Inbar M, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Virology; 40(4):854-859 1970

SV40-induced structural changes in the surface membrane of 3T3 cells were measured by agglutination with the carbohydrate-binding protein concanavalin A. It was demonstrated that cell replication was necessary for these surface membrane changes to occur and for the loss of this change to occur in abortively-transformed cells. Agglutination with concanavalin A occurred only in multiplying cultures and required at least 1 cell generation and a density of about 105 cells/cm2. The percentage of cells containing T antigen in multiplying cultures was of the same order of magnitude as the 50% of cells that formed aggregates 6 days after SV40 infection. About 90% of the agglutinated cells contained T antigen. It is indicated that abortive transformation can produce the same surface membrane changes as hereditary transformation.

247. SACCHARIDES ON TERATOCARCINOMA CELL PLASMA MEMBRANES. THEIR INVESTIGATION WITH RADIOACTIVELY LABELED LECTINS.

Gachelin G, Buc-Caron MH, Lis H, Sharon N
Departement de Biologie Moleculaire, Institut Pasteur, 25 Rue du Dr. Roux, 75015 Paris, France
Biochim Biophys Acta; 436(4):825-832 1976

The interaction of various lectins with the surface of three clonal cell lines from teratocarcinoma (F9, PCC3 and Pys-2) were examined in an attempt to identify any specific cell markers. The differentiation of primitive embryonal carcinoma cells into parietal yolk sac cells was accompanied by changes in cell surface saccharides. These changes consisted of a marked decrease in the total number of binding sites for the L-fucose specific lectin of Lotus tetragonolobus and a large increase in the total number of binding sites for wax bean agglutinin. It is suggested that these differences can be used as markers in the study of early embryonic differentiation. No agglutination of primitive embryonal carcinoma cells or of parietal yolk sac cells by low concentrations (10 microg/ml) of concanavalin A, soybean agglutinin or the fucose binding proteins was observed. These cells do not behave as typical tumor cells. (25 refs)

248. THE TRANSFORMED CELL SURFACE: AN ANALYSIS OF THE INCREASED LECTIN AGGLUTINABILITY AND THE CONCEPT OF GROWTH CONTROL BY SURFACE PROTEASES.

Talmadge KW, Noonan KD, Burger MM
Biocent., Univ. Basel, Switzerland
Cold Spring Harbor Conf Cell Proliferation; 1:313-325 1974

Theories of lectin-mediated agglutination of virus-transformed cells are reviewed and experimental data are presented on the relevance of receptor site mobility and membrane proteolytic activity for the agglutination process. Treatment with glutaraldehyde prevented the agglutinability of polyoma virus-transformed 3T3 cells (Py3T3) but did not interfere with lectin binding. Temperature-dependence studies for agglutination by concanavalin A indicated that agglutination does not occur below the freezing point for lipids. While suggesting that agglutination may require some freedom of mobility, these results did not demonstrate whether increased agglutinability in transformed cells is caused by an increased mobility of macromolecules within the membrane. The effects of mild protease treatment of untransformed mouse and chicken fibroblasts suggested that a proteolytic activity associated with the surface membrane of transformed cells might be important in the release from growth control: protease treatment led to a transient agglutinability of the untransformed cells and a transient release from density-dependent inhibition of growth. Studies of the interaction of Py3T3 cells and the protease inhibitor ovomucoid revealed that cell growth was significantly inhibited by polyacrylamide bead-coupled ovomucoid containing 100-420 microg protein/106 beads. In the presence of the ovomucoid beads, the Py3T3 cells stopped growing at a density of 2 x 105 cells/cm2. The growth inhibition did not involve uptake of ovomucoid and was apparently related to a critical event in the plasma membrane. A hypothetical cascading system of membrane-attached proteases is presented. The system may explain how different treatments of cell cultures all lead to growth promotion and how activated trigger increased agglutinability.

249. STRUCTURES AND ORGANIZATION OF CELL SURFACE GLYCOLIPIDS: DEPENDENCY ON CELL GROWTH AND MALIGNANT TRANSFORMATION.

Hakomori SI
Sch. Public Health, Univ. Washington, Seattle
Biochim Biophys Acta; 417(1):55-89 1975

A discussion of interrelationships between chemical, enzymatic, and organizational changes of glycolipids, and a correlation of those changes as a function of tumor cell characteristics is presented. Considerations of the chemical change of glycolipid composition in transformed cells include the universal changes observed, simplification of glycolipid patterns due to blocked syntheses, various other changes due to transformation, the change in blood group glycolipids and fucolipids associated with malignant transformation, and chemical patterns and syntheses in tumor cells. The enzymatic basis of the chemical change of glycolipids is investigated using glycosyltransferases and glycosylhydrolases . Studies reveal contact-dependent enhancement of glycolipid synthesis; this is assumed due to the presence of cell surface glycosyltransferases. Investigations on the organization of glycolipids, glycoprotein, and other membrane components reveal the relationship of galactoprotein a to growth control and cell contact, the qualitative differences of the lectin-binding protein in normal and transformed cells, the greater exposure of glycolipids in transformed cells, the presence of a specific sialyl-galactosyl or sialyl- N-acetyl-galactosaminyl protein in transformed cell surfaces, and the presence of

specific surface labels for specific glycolipids. Three general categories of changes are observed: (1) changes in glycolipid composition and synthesis, (2) absence of cell contact-dependent enhancement of glycolipid synthesis, and (3) deletion of a high molecular weight galactoprotein a and exhibition of a higher extent of glycolipid exposure on transformed cells. (121 refs)

250. THE CELL SURFACE.

Berlin RD, Oliver JM, Ukena TE, Yin HH
Univ. Connecticut Health Cent., Farmington, CT
N Engl J Med; 292(10):515-520 1975

Experimental evidence from studies of cell surface topography, using cultured fibroblasts, lymphocytes, and phagocytic cells that demonstrates characteristic, nonrandom distribution patterns for several different membrane components is reviewed. A proposal is made that structures extrinsic to the membrane, (e.g, microtubules) are important in determining its properties. It is postulated that for the membrane to exist in a state of lowest free energy, lipids and proteins must be arranged so as to maximize hydrophobic bond formation with the lipid, and hydrophilic groups interact with the aqueous environment; this model is designated the "fluid mosaic model". It allows considerable compositional differences between membranes, the accommodation of proteins thought to extend through the membrane, and the prediction of no long-range forces intrinsic to the membranes. Experiments on membranes illustrate the existence of a random distribution of proteins free to move over the surface (homogenous topography), plus a nonrandom distribution (heterogeneous topography). Topographic changes induced in cancer cells imply the existence of forces limiting free diffusion of membrane proteins, and suggest differences in the nature or effectiveness of restraining or limiting factors. Data on lectin binding to the cell surface confirm that ligand-receptor complexes are mobile on cell surfaces, and indicate that forces other than free diffusion are probably involved in the ordering of surface topography, including agents acting directly on the membrane and those affecting intracellular processes. It is noted that nonrandom movements can occur on cell surfaces even in the absence of exogenous ligands, as observed in heterogeneity induced by phagocytosis; a similar segregation of membrane proteins is suggested occurring during extrusion of nuclei from maturing reticulocytes. Evidence presented in support of surface topography regulated by the interaction of the plasma membrane with a specific class of cellular constituents resembling microtubular proteins suggests that microtubular proteins are essential for the heterogeneous topography induced by phagocytosis. Pharmacologic evidence implicates only a microtubular protein, and not necessarily the morphologically recognizable microtubule. However, it is proposed that a reversible association of microtubules, and perhaps microfilaments, with membrane components is induced by ligand or particle contact, and is involved in the movement of surface proteins. (16 refs)

251. RAPID ADSORPTION OF A FOETAL CALF SERUM COMPONENT BY MAMMALIAN CELLS IN CULTURE: A POTENTIAL SOURCE OF ARTIFACTS IN STUDIES OF ANTISERA TO CELL-SPECIFIC ANTIGENS.

Kerbel RS, Blakeslee D
Division of Cancer Research, Department of Pathology, Queen's University, Kingston, Ontario, Canada
Immunology; 31(6):881-891 1976

Injection of CBA mice with mitogen stimulated (either LPS or con A) CBA lymphocytes which had been cultured for 3 days in the presence of foetal calf serum (FCS) led to the production of antisera which reacted strongly with virtually all types of mammalian cells, including human, whether normal or malignant, provided they had been cultured in FCS-containing media. Reactivity was detected by sensitive immunological assays such as complement/dependent cytotoxicity using rabbit complement (but not using guinea-pig complement), or EA-rosette inhibition of Fc receptor-bearing cells. The antisera did not react with fresh normal lymphoid cells or ascites tumour cells; however, these same cell populations became fully susceptible to the cytotoxic effects of the antisera after as little as 4 h incubation at 37 degrees in the presence of FCS. Cells incubated without FCS or with FCS at 0 degrees were not affected. The antisera reacted with FCS to form a single band on Ouchterlony double-diffusion plates. On immunoelectrophoresis the reactive antigen appeared to migrate in the alpha-globulin region of serum proteins. These observations suggest that FCS may be a source of potentially serious misinterpretations in immunological studies of cell-associated antigens using antisera produced by the injection of cells grown in FCS-containing cultures. Examples of artifacts arising from the use of FCS in certain systems, eg the preparation of alloantisera using cultured tumour cells vs fresh non-cultured lymphoid cells, are described. (Author Abstract)

252. MECHANISM OF CELL ENTRY AND TOXICITY OF AN AFFINITY-PURIFIED LECTIN FROM RICINUS COMMUNIS AND ITS DIFFERENTIAL EFFECTS ON NORMAL AND VIRUS-TRANSFORMED FIBROBLASTS

Nicolson GL, Lacorbiere M, Hunter TR
Salk Inst. Biol. Stud., San Diego, Calif.
Cancer Res; 35(1):144-155 1975

An affinity-purified plant lectin from Ricinus communis (RCAII) was used to investigate the mechanism of Ricinus communis lectin toxicity and also its effects on normal and Simian virus 40, (SV40)-transformed cells. Cells were grown to subconfluency, treated with lectin solution for various times at 37 C, incubated with 3H-leucine (3 microC/ml) for one hr, and protein was precipitated. RCAII suppressed cell protein synthesis in the transformed line at lower concentrations (1/50 and 1/100) compared to the 3T3 line; these effects were blocked by the RCAII inhibitors, D-galactose or lactose. RNA and DNA synthesis and L-leucine transport were relatively unaffected by RCAII concentrations (greater than 1 microg/ml) that completely suppressed protein synthesis in both cell lines. The kinetics of 125I-RCAII binding to intact cells (saturation in approximately 5-10 min, even at 4 C) compared to the time required to inhibit cell protein synthesis (greater than 80% inhibition in approximately 60 min at 37 C) indicated that events subsequent to cell binding were necessary for the action of RCAII. That RCAII acts directly on protein synthesis after cell entry was confirmed with rabbit reticulocyte and mouse Krebs II ascites S30 cell-free protein synthesis systems. Concentrations of RCAII (0.1-1 microg/ml) that inhibit cell protein synthesis in 90 min act within 1-3 min to suppress cell-free protein synthesis. The saccharide prevention of RCAII inhibition of cell, but not cell-free, protein synthesis indicated that different sites may be involved in cell binding and inhibition of protein synthesis. The results suggest that RCAII-mediated toxicity occurs by the following sequence of events: 1) cell surface binding; 2) lectin-induced surface clustering of RCAII receptors; 3) endocytosis of RCAII; 4) release of RCA from inside endocytotic vesicles into the cell cytoplasm; and 5) direct RCAII interaction and inactivation of protein synthesis.

253. LOCALIZATION OF THE BINDING SITES FOR THE RICINUS COMMUNIS, AGARICUS BISPORUS AND WHEAT GERM LECTINS ON HUMAN ERYTHROCYTE MEMBRANES.

Triche TJ, Tillack TW, Kornfeld S
Washington Univ. Sch. Medicine, St. Louis, MO 63110
Biochim Biophys Acta; 394:540-549 1975

Freeze-etch electron microscopy was used to localize the binding sites for the Ricinus communis, Agaricus bisporus, and wheat germ lectins on human RBC membranes and to determine the relation of these different glycoprotein receptors to the intramembranous particles. A bisporus lectin and ferritin conjugates of wheat germ agglutinin showed a distribution that correlates exactly with the intramembranous particles at all lectin conc tested. Lectin binding sites are located on the major sialoglycoprotein of the membrane. The R. Communis agglutinin-ferritin conjugate showed a close correlation with the intramembranous particles at low lectin conc and a poor correlation at high lectin conc. High conc resulted in virtually complete coating of the surface of trypsinized ghosts which displayed marked aggregation of the intramembranous particles. It is concluded that the intramembranous particles of RBC membranes contain at least two glycoproteins and that some membrane lectin receptors are not associated with the intramembranous particles.

254. CELL-SURFACE CHANGES IN A RICINUS COMMUNIS TOXIN (RICIN)-RESISTANT VARIANT OF A MURINE LYMPHOMA.

Robbins JC, Hyman R, Stallings V, Nicolson GL
Dept of Cancer Biology, The Salk Inst for Biological Studies, San Diego, Ca 92112.
J Natl Cancer Inst; 58(4):1027-1033 1977

A variant of the murine lymphoma cell line BW5147 that was 250 times more resistant than the parent to Ricinus communis II agglutinin (RCA2, ricin) toxicity (measured in the absence of serum) was selected by repeated exposure of cells to increasing concentrations of the lectin. Quantitative binding of the lectin, however, was decreased by only 30-40% in the variant. In contrast with several reported lectin-resistant variants, most surface glycoproteins on the parental and variant cell surfaces were similar, as judged by electrophoresis after lactoperoxidase-catalyzed iodination and RCA1-affinity chromatography. Surface studies showed that an RCA1- and RCA2-binding protein of about 80,000 daltons on the surfaces of parental cells is altered on the variant cells to a form with a lower apparent molecular weight. We suggested that this protein is important for entry of RCA2 molecules in parental cells, but that its altered form on the variant cells no longer mediates efficient RCA2 uptake, thus imparting toxin resistance. In addition, a protein of approximately 35,000 daltons, which does not bind RCA1, is weakly lactoperoxidase-iodinated on parental but not variant cells. (27 Refs). (Author Abstract)

255. RECEPTORS FOR HELIX POMATIA A HAEMAGGLUTININ ON LEUKAEMIC LYMPHOCYTES FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL).

Hellstrom U, Mellstedt H, Perlmann P, Holm G, Pettersson D
Dept. Immunology, Univ. Stockholm, Fack, S-104 05 Stockholm, Sweden
Clin Exp Immunol; 26(2):196-203 1976

Blood lymphocytes from 13 patients with chronic lymphocytic leukemia (CLL) were studied for surface-bound Ig (SIg), Fc receptors (EA rosettes), receptors for sheep RBC (E rosettes) and receptors for Helix pomatia A hemagglutinin (HP), a carbohydrate-binding protein with specificity for N-acetyl-D-galactosamine and related sugars. Eleven patients were untreated or in relapse for more than 2 yr, while two were in clinical remission after recent cytostatic therapy. Fluorescein-labeled HP bound to subpopulations of human peripheral blood lymphocytes (PBL) treated with neuraminidase. In normal peripheral blood, HP bound to the T lymphocytes while the majority of the B cells bearing surface-bound immunoglobulin did not have receptors for HP. In untreated CLL, HP bound to 90%-100% of the neuraminidase-treated PBL. Almost all of the SIg-positive cells in CLL patients also had receptors for HP. Two groups of patients were found: in one the total fraction of SIg+ cells was 50% and about 30% of these lost their Ig during incubation at 37 C. No such loss of SIg was revealed in the remaining patients where total SIg+ fraction was approx 70%. These patients usually had higher blood lymphocyte counts, probably reflecting a more advanced disease. CLL patients in remission with low numbers of leukemic cells also had low numbers of blood lymphocytes carrying both SIg and HP-receptors. It is concluded that leukemic cells carry both HP receptors and SIg. Testing of this combination therefore provides a valuable new tool for monitoring patients with CLL. (27 refs)

256. SACCHARIDES ON TERATOCARCINOMA CELL PLASMA MEMBRANES: THEIR INVESTIGATION WITH RADIOACTIVELY LABELLED LECTINS.

Gachelin G, Buc-Caron MH, Lis H, Sharon N
Department de Biologie Moleculaire, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris
Arch Int Hist Sci (Paris); 436(4):825-832 1976

We have studied the interaction of five lectins differing in their sugar specificity, with the surface of clonal cell lines derived from transplantable murine teratocarcinoma. The results show that the differentiation from primitive embryonal carcinoma cells into parietal yolk sac cells is accompanied by changes in cell surface saccharides. These changes consist of a marked decrease in the total number of binding sites for the L-fucose-specific lectin of Lotus tetragonolobus and a large increase in the total number of binding sites for wax bean agglutinin. It is suggested that these differences can be used as markers in the study of this early embryonic differentiation. No agglutination of primitive embryonal carcinoma cells or of parietal yolk sac cells by low concentrations (10 mu g/ml) of concanavalin A, soybean agglutinin or the fucose binding proteins was observed. (Author Abstract)

257. ISOLATION OF CELL SURFACE GLYCOPROTEIN WHEAT GERM AGGLUTININ RECEPTORS FROM HELA CELL PLASMA MEMBRANES (MEETING ABSTRACT).

Kramer RH, Canellakis ES
Dept. of Pharmacology, Yale Univ Sch. of Med., New Haven, CT 06510
Proc Am Assoc Cancer Res; 18:56 1977

Many tumor cells can undergo differential lectin induced agglutination and cytotoxicity as compared to their normal, non-neoplastic counterparts. It was found that wheat germ agglutinin (WGA) will produce agglutination, inhibition of amino acid transport, and cell death in HeLa cells. Using affinity chromatography, a number of cell surface WGA-reactive glycoproteins from HeLa cells have been isolated and identified. Cells were labeled by lactoperoxidase catalyzed ^{125}I-iodination and the purified plasma membranes were solubilized and chromatographed on WGA-agarose columns according to the procedure of Kalane et al (BBA 426: 357, 1976). The non-bound fraction from the WGA column, as analyzed by SDS-polyacrylamide gel electrophoresis, was found to contain all of the major Coomassie blue staining polypeptides

as well as several ^{125}I-labelled components. N-Acetyl glucosamine, a haptenic inhibitor of WGA, eluted seven major ^{125}I-labelled proteins (MW approx 40,000- approx 300,000) which were poorly if at all stained with Coomassie blue. In addition, four of these polypeptides, with MW's of 72,000, 127,000, 165,000, and 230,000, could be metabolically labelled with (^{3}H)-D-glucosamine. These findings indicate that there are multiple cell surface WGA-reactive glycoproteins which may be involved in WGA mediated cytotoxicity. (Author Abstract)

258. DIRECT TOXIC EFFECTS OF IMMUNOPOTENTIATORS ON MONOCYTIC, MYELOMONOCYTIC AND HISTIOCYTIC OR MACROPHAGE TUMOR CELLS IN CULTURE.

Ralph P, Nakoinz I
Sloan-Kettering Institute for Cancer Research, Rye, New York 10580
Cancer Res; 37(2):546-550 1977

Four murine monocyte, myelomonocyte, and histiocyte or macrophage tumor cell lines adapted to culture were growth inhibited by one or more of the following macrophage-activating substances: Mycobacterium bovis, Bacillus Calmette-Guerin strain, zymosan, lipopolysaccharide, and dextran sulfate, as well as tuberculin purified protein derivative, but not latex beads. Lipopolysaccharide was effective with one line at 4 ng/ml. All four lines actively phagocytosed zymosan and latex beads. In many cases the growth inhibition was apparently immediate but only cytostatic, and cell proliferation resumed upon removal of the drug. Bacillus Calmette-Guerin, live or boiled, was toxic to some of the tumor lines. Synthesis of lysozyme by all the cell lines in the monocyte series and production of granulocyte colony-stimulating factor by the myelomonocytic leukemia were not inhibited during several days of zero growth conditions in the presence of drugs. Since these agents had no direct effect on other hematopoietic tumor types (myeloma, T-lymphoma, mastocytoma) at the same or up to 10^4 higher concentrations, it is proposed that the sensitive tumors retain specific receptors for immunostimulants, either at the cell surface or within the cell in the case of phagocytosable particles. The binding of these agents to physiological receptors leads to stimulation and mitogenesis in normal macrophages and lymphocytes but leads to growth inhibition without affecting differentiated functions in the corresponding tumor lines. (Author Abstract)

259. COMPARISON OF EARLY EMBRYONIC AND DIFFERENTIATING CELL SURFACES: INTERACTION OF LECTINS WITH PLASMA MEMBRANE COMPONENTS.

Zalik SE, Cook GM
Dept. Zoology, Univ. Alberta, Edmonton, Alberta, Canada T6G 2E1
Biochim Biophys Acta; 419(1):119-136 1976

Cells of the unincubated and those of primitive streak chick blastoderm, which were preparing for or were involved in morphogenetic movements, were agglutinated by wheat germ agglutinin, Ricinus communis agglutinin and concanavalin A, but not by fucose-binding protein. Agglutination of these cells with soybean agglutinin occurred only after neuraminidase treatment, while that induced by concanavalin A, wheat germ and Ricinus communis agglutinins was not affected. Trypsin treatment of blastoderm cells had no effect on lectin-mediated agglutination. Cells derived from 10- and 12-day differentiating chick liver were agglutinated by wheat germ agglutinin only after trypsinization. Mechanically dissociated embryonic liver cells, which were not agglutinated, bound more ^{3}H-labeled wheat germ agglutinin per cell than trypsinized cells. This suggested that during differentiation, there might be a spatial reorganization of wheat germ agglutinin receptors within the plasma membrane. Membranes isolated from the above cell types were examined by analytical polyacrylamide gel isoelectric focusing and, in combination was affinity chromatography using wheat germ agglutinin conjugated to agarose, membrane material in the differentiating liver membrane, which bound to this lectin, was identified.

260. DIFFERENTIAL EFFECT OF TUNICAMYCIN ON THE VIABILITY AND CELL SURFACE PROPERTIES OF NORMAL AND TRANSFORMED 3T3 CELLS (MEETING ABSTRACT).

Duksin D, Bornstein P
Dept of Biochemistry Univ of Washington, Seattle, WA 98195
Fed Proc; 36(3):745 1977

Normal and virally transformed 3T3 cells were treated with tunicamycin (TM), an inhibitor of protein glycosylation. TM (1 mug/ml) caused detachment and death of SV40-3T3 and Py-3T3 cells within 24 hrs; these effects were not seen with 3T3 cells or with embryonic chick tendon fibroblasts. However, the proliferation of 3T3 cells was inhibited by TM and, after several days, there occurred a drastic change from an epithelioid to an abnormally elongated shape. Both inhibition of growth and the morphological changes were reversible. One explanation for these observations is that impaired secretion or the synthesis of carbohydrate-poor glycoproteins in the presence of TM may affect a number of cell surface properties, including cell attachment and shape. In support of this hypothesis, a marked (approx 100 fold) decrease in Con-A agglutinability was observed in virally transformed 3T3 cells grown in the presence of TM. Agglutination by wheat germ and soybean agglutinins was unaffected. The agglutinability of trypsinized 3T3 cells by all three lectins was unchanged by TM. These results suggest that TM interferes with the insertion or function of a specific Con-A binding protein on the surface of cells. The more marked effect of TM on transformed 3T3 cells may result from reduced synthesis, more rapid turnover or improper function of cell surface glycoproteins. (Author Abstract)

261. THE FORMATION OF VARIANTS WITH A REVERSION OF PROPERTIES OF TRANSFORMED CELLS. III. REVERSION OF THE STRUCTURE OF THE CELL SURFACE MEMBRANE.

Inbar M, Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovoth, Israel
Int J Cancer; 4(5):690-696 1969

The formation of variants from polyoma virus (PV)-transformed hamster embryo cells with a reversion of in vitro properties of transformation is associated with a partial or complete loss of agglutinability by the carbohydrate-binding protein concanavalin A (Con A). Treatment of variants with trypsin resulted in restoration of previously-lost agglutinability. Since the formation of variants was not associated with a loss of ability to synthesize the virus-specific nuclear tumor (T) antigen, it is suggested that the synthesis of T antigen is insufficient to prevent reversion of cell surface architecture. Compared with parental transformed cells, variants with a partial or complete loss of agglutinability showed the same decrease in cloning efficiency in fluid medium and soft agar and in saturation density. Apparently both complete and partial reversion of Con A sites to the cryptic form can account for the same degree of reversion of in vitro properties. It is concluded that there is some minimal number of exposed sites for Con A which must be exceeded for the cells to express the properties of transformation in vitro.

262. ANALYSIS OF LYMPHOCYTE STIMULATION BY LECTINS AND LECTIN DERIVATIVES.

Cunningham BA
Rockefeller U., New York, N. Y.
Biomed Perspect Agglutinins Invertebrate Plant Origins; 234:219-225 1974

The mitogenic lectins, particularly concanavalin A (Con A) have been used to study the initial events required at the cell surface for the stimulation of mitosis in lymphocytes. Since the mechanisms by which Con A exerts its various biological activities on lymphocytes may depend on its molecular size, shape, organization of subunits, and the number and type of binding sites, the structure of the protein must be known in detail. Treatment of Con A with succinic anhydride produces succinyl-Con A, a lectin which at pH 7.4 has the same specificity but differs in charge and has half the valence of native Con A. Both lectins bind to lymphocytes and erythrocytes, but with mouse lymphocytes Con A can exhibit two antagonistic properties not shown by succinyl-Con A: the ability to induce cap formation with its own receptors; and the ability to restrict the mobility of ligand receptor complexes such as anti-Ig plus cell receptors. The combination of anti-Con A and bound succinyl-Con A has properties similar to those of Con A. These abilities are probably more critically dependent on valence than on the net charge. Both lectins are equally effective mitogens, they both show maximal stimulation in concentrations as dilute as 6 microg/ml. Observations indicate that the process of capping is not required for mitogen stimulation. The increased negative charge of the succinyl derivative may be more critical than its valence in its ability to prevent cell death. The valence of the lectin appears to play an important role in the modulation of the mobility of lymphocyte surface receptors, but the net charge may be equally important in the inhibition of mitogenesis at high lectin concentrations. Mitogenic lectins, unlike non-mitogenic lectins, may be able to bind surface receptors which are attached to a postulated system of colchicine-binding proteins.

263. REACTION OF CONCANAVALIN A WITH ALKALINE PHOSPHATASES EXTRACTED FROM VARIOUS HUMAN TISSUE SOURCES.

Chang CH, Angellis D, Fishman WH
Tufts Univ. Sch. Medicine, Boston, MA 02111
Mol Cell Biochem; 9(1):55-57 1975

A specific reaction between concanavalin A (Con A) and a fraction of alkaline phosphatases extracted from various human tissues was demonstrated by an electrophoretic retardation technique and Con A-affinity chromatography. This phenomenon was also observed with highly purified placental alkaline phosphatase with a specific activity of 200 micromole phenol mg protein/min. In light of the well-established specificity of Con A reactivity toward the carbohydrate moiety, the results indicate that the reacting human alkaline phosphatases may contain branched polysaccharides with a terminal alpha-D-mannopyranose, alpha-D-glucopyranose, D-fructofuranose, their glucosides, or sterically related structures. It remains to be established whether the alkaline phosphatase in situ on the cell surface may also have exposed sugar residues capable of binding Con A freely. However, with regard to attempts to isolate and identify the Con A "receptor" from the solubilized fractions of cell membrane by Con A-affinity chromatography or other related procedures, future efforts should take into consideration the membrane-associated alkaline phosphatase.

264. SURFACE ALTERATIONS IN CALF LYMPHOCYTES OXIDIZED BY SODIUM PERIODATE.

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Division of Hematology And Oncology, Department of Medicine, The Jewish Hospital of St. Louis, Washington University School of Medicine, St. Louis, Missouri 63110
J Biol Chem; 251(7):1864-1870 1976

In order to investigate alterations in surface structure in transformed lymphocytes, calf submandibular lymph node cell suspensions were oxidized with $NaIO_4$. Oxidized lymphocytes were morphologically transformed and had higher rates of DNA synthesis by 2 days after treatment. These results were prevented by reduction of the cell suspension with $NaBH_4$, or by neuraminidase treatment of cells prior to oxidation. The amount of ^{125}I-labeled Agaricus bisporus lectin bound to cells immediately after oxidation and the affinity constant for binding were increased over 2-fold, while cells immediately following oxidation and reduction showed decreased receptors with still higher affinity for the lectin compared to untreated cells. The amount of Phaseolus vulgaris lectin bound to oxidized cells was also increased, but affinity was unchanged. Immediately following oxidation and reduction, these receptor sites were unchanged in number and affinity from untreated cells. In contrast, the number and affinity of receptors for concanavalin A were not changed immediately after oxidation or oxidation and reduction. In order to define the extent of compositional changes in surface glycoprotein receptors, plasma membranes were isolated from frozen calf submandibular lymph nodes. Compared to untreated plasma membranes, oxidized membranes had similar contents of galactose, mannose, N-acetylglucosamine , N-acetylgalactosamine ,fucose, and amino acids. Sialic acid content of oxidized membranes was reduced when measured by thiobarbituric acid assay. Sialic acids of untreated plasma membranes co-chromatographed with N-glycolylneuraminic acid and N-acetylneuraminic acid , while those of oxidized membranes co-chromatographed with N-glycolylneuraminic acid and 5-acetamido-3,5-dideoxy-L-arabino-7-aldehydo-2-heptulosonic acid. Therefore, specific surface conformational changes in certain classes of membrane glycoproteins are associated with mild Malapradian oxidation of membrane sialic acids. The temporally precede $NaIO_4$-induced transformation of calf lymphocytes. This is consistent with an hypothesis of membrane-mediated stimulation of lymphocyte transformation.

265. AN ANALYSIS OF LECTIN-INITIATED CELL AGGLUTINATION IN A SERIES OF CHO SUBCLONES WHICH RESPOND MORPHOLOGICALLY TO GROWTH IN DIBUTYRYL CYCLIC AMP.

Van Veen J, Roberts RM, Noonan KD
J. Hillis Miller Health Center, Univ. Florida, Gainesville, FL 32610
J Cell Biol; 70(1):204-216 1976

The molecular basis of the agglutinability of CHO subclones that respond differentially in terms of morphology and surface architecture in the presence of dB-cAMP was investigated. It was demonstrated that the agglutinability of these subclones with both wheat germ agglutinin (WGA) and concanavalin A (Con A) probably depends on the free lateral mobility of the lectin receptor sites in the membrane plane. The nonagglutinable surface architecture seems to depend on the presence in the membrane of a proteaselabile peptide(s), which appears to be distinct from the lectin receptors, as well as on continuous protein and RNA synthesis. This dependence on continuous transcription and translation may be related to maintenance of the protease-labile peptide(s) in such a state as to restrict mobility of the lectin receptors. The surface architecture defined as nonagglutinable also depends on the

state of polymerization of the intracellular microtubules and microfilaments. These microskeletal elements probably anchor the lectin receptors in such a manner as to restrict their mobility and thereby reduce the relative agglutinability of a cell line. Control of the free mobility of both the Con A and WGA receptor sites is thought to be dependent on two constraints, one applied by protease-labile ("surface") membrane components and the other by components of the intracellular microskeletal system.

266. MECHANISM OF CELL ENTRY AND TOXICITY OF AN AFFINITY-PURIFIED LECTIN FROM RICINUS COMMUNIS AND ITS DIFFERENTIAL EFFECTS ON NORMAL AND VIRUS-TRANSFORMED FIBROBLASTS.

Nicolson GL, Lacorbiere M, Hunter TR
Dept. Biol., Salk Inst., Biol. Studies, San Diego, Calif.
Cancer Res; 35(1):144-155 1975

An affinity-purified lectin from Ricinus communis (castor bean) (RCA2) exhibited differential toxicity toward SV40 virus-transformed 3T3 BALB/c clone A31 mouse fibroblasts in vitro. RCA2 suppressed cell protein synthesis in the SV40 transformed 3T3 fibroblasts at 1/50 to 1/100 of the conc required for the same effect in normal 3T3 fibroblasts. This effect was inhibited by the RCA2 inhibitors, D-galactose or lactose. RNA and DNA synthesis were relatively unaffected by RCA2 conc. (greater than 1 ug/ml) causing complete inhibition of protein synthesis in both cell lines. The inhibition of protein synthesis required incubation periods of greater than 1 hr, although RCA2 was shown to bind completely in 5-10 min, even at 4. In 10 min labeling experiments with ^{125}I-RCA2 (1 ug/ml), the cell-bound lectin could be removed up to 15 min after binding without causing subsequent inhibition of protein synthesis. Progressively less RCA2 could be removed after longer incubation periods, and only 20-30% could be removed by washing after 1 hr. Inhibition of protein synthesis also occurred after incubation periods of greater than 15 min. Ferritin-conjugated RCA2 (FeRCA2) bound in less than 10 min to SV40-transformed 3T3 cell surfaces and was blocked by lactose. The complex was found randomly on the cell surfaces after 10 min incubation at 4, but after washing and incubation at 37 the FeRCA2 induced clustering of its receptors in 15-30 min and eventually induced endocytosis (30-60 min). Further incubation resulted in FeRCA2 localization inside endocytotic vesicles and in the cytoplasm. Rabbit reticulocyte and mouse Krebs II ascites S30 cell-free protein synthesis systems were inhibited in 1-3 min by RCA2 (0.1-1.0 ug/ml). Saccharides did not inhibit cell-free protein synthesis, suggesting different sites of action of RCA2 inside and outside the cells, for membrane binding and protein synthesis inhibition. RCA2-mediated cytotoxicity occurred in the sequence of 1) cell surface binding, 2) lectin-induced clustering of cell surface RCA2 collectors, 3) endocytosis of RCA2, 4) release of RCA2 from inside endocytotic vesicles into the cytoplasm, and 5) direct RCA2 interaction and inactivation of protein synthesis. The similarity between the mode of action of RCA2 and ricin is discussed. (45 refs)

267. RELATIONSHIP BETWEEN BIOCHEMICAL AND IMMUNOLOGICAL CHANGES. (PP. 229-238)

Defendi V
Cell Surfaces and Malignancy. John E Fogarty International Center for Advanced Study in Health Sciences and Natl Insts Health Bethesda MD, September 11-13 1974, 1975.

The author reviews studies on the biochemical changes of transformed cells that can be related to modification of antigenicity. The antigenic changes at the tumor cell surface can be classified as being associated with general tumor or tumor-associated antigens, specific tumor antigens, and private tumor antigens. In the first group the antigens are shared by tumor cells independent of the causative agent and are probably associated with membrane changes characteristic of rapidly growing cell populations. These antigens are the most difficult to identify. Specific tumor antigens are those characteristic of either tumors produced by viruses or tumors of a definite histiotype. Private antigens are distinctive for each tumor. Most of the biochemical and structural studies of tumor cell membranes have been conducted with cells transformed in vitro by RNA or DNA tumor viruses. Some of the new antigens found are viral products, such as the glycoproteins of the coat of RNA tumor viruses. Whether these antigens have any direct role in the observed plasma membrane changes is not clear. Other tumor-specific antigens, such as the transplantation antigen (TSTA), appear to be virus-specific, even though they are not part of the virion structure per se. TSTA is an expression of viral infection and not necessarily of the malignant phenotype, since it is also found in primary infected cultures of permissive cells that are not programmed to become tumor cells. Virus-induced tumor cells also have TSTA's that are shared with antigens present in embryos during limited stages of their development. There are four biochemical changes that could be relevant to modification of antigenicity: (a) changes in agglutinin receptor sites that favor cell agglutination by plant lectins; (b) an increase of a high molecular wt sialic-fucose labeled glycopeptide in solubilized cell surface fractions after trypsin and pronase treatment with a corresponding increase of specific sialyl transferase; (c) a simplification of glycosphingolipid components to small or homologous ones containing fewer carbohydrates, including sialic acid; and (d) the loss of a group of glycoproteins or proteins of different molecular wt from the surface of transformed cells.

B. Relationships of Immunoglobulin Receptors to Differentiation, Cytotoxicity and Tolerance Induction

268. ISOLATION AND CHARACTERIZATION OF FC SURFACE RECEPTORS FOR HUMAN IGE FROM CULTURED HUMAN LYMPHOBLASTOID CELLS WIL-2WT (MEETING ABSTRACT).

Meinke GC, Spiegelberg HL
Scripps Clinic and Res Fndn, La Jolla, CA 92037
Fed Proc; 36(3):1186 1977

Wil-2WT cells were labeled either enzymatically with ^{125}I by the lactoperoxidase method or biosynthetically by culturing in the presence of ^{14}C-leucine. Labeled cells were washed in cold reticulocyte standard buffer (RSB) and lysed with 0.5% NP-40 in RSB, pH 7.4 at 4 degrees for 15-30 min in the presence of protease inhibitors. After centrifugation at 1000 g to remove nuclei, the supernatant was spun at 100,000 g for 1-2 hours and dialyzed against 0.5% NP-40 PBS pH 7.2. Equal volumes of cell lysate were passed over IgE and/or IgG Sepharose immunoadsorbent columns equilibrated in 0.5% NP-40 PBS. Approximately twice as many counts (0.1-0.5% of the total counts) were eluted from IgE immunoadsorbent columns compared to IgG columns. TCA precipitates of eluates from immunoadsorbent columns were analyzed by 10% SDS-PAGE. The majority of the radioactivity of the IgE column eluates migrated in the 45-55,000 MW region. Protein from a lysate of 2×10^{9} cells isolated on the IgE column was injected into a goat in CFA. The antiserum obtained did not react with human serum proteins. After absorption with SRBC, ox-RBC and cultured human lymphocytes RPMI 4098 which do not have Fc IgE receptors, the antisera inhibited rosette formation between IgE coated red cells and Wil-2WT cells in dilutions up to 1:20. In contrast the preimmune serum did not inhibit rosette formation even at a dilution of 1:2. (Author Abstract)

69. A FUNCTIONAL COMPARISON OF HUMAN FC-RECEPTOR-BEARING LYMPHOCYTES ACTIVE IN NATURAL CYTOTOXICITY AND ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY.

Kay HD, Bonnard GD, West WH, Herberman RB
Lab. Immunodiagnosis, NCI, NIH, Bethesda, MD 20014
J Immunol; 118(6):2058-2066 1977

Natural cytotoxicity against the K-562 myeloid cell line and antibody-dependent cellular cytotoxicity (ADCC) against sensitized Chang liver cells were investigated using the same preparations of normal human peripheral blood lymphocytes as effector cells. Both types of cytotoxicity were exhibited by lymphocytes with receptors for sheep RBC and for the Fc portion of immunoglobulin G (IgG). Up to 80% of the natural cytotoxicity and ADCC activities were recovered in E-rosette cells separated under optimal conditions. In addition, both types of activity were greatly depressed by brief exposure to ammonium chloride solutions and both activities returned to normal after 24 hr at 24 C. When lymphocytes with Fc receptors were adsorbed onto plastic surfaces coated with a monolayer of immobilized antigen-antibody complexes, both natural and ADCC activities were simultaneously decreased. This decrease was blocked by incubation of Staphylococcal protein A on the antigen-antibody monolayers prior to lymphocyte addition. When protein A was added to the ADCC incubation mixtures (0.01 to 100 g/ml), ADCC was significantly inhibited. When lymphocytes were treated with purified trypsin or alpha-chymotrypsin at (0.01 to 10 mg/ml), natural cytotoxicity was inhibited whereas ADCC was unaffected. The data indicate that effector cells in natural cytotoxic and ADCC reactions are overlapping subpopulations of lymphocytes probably derived from T cells. The cytolytic mechanisms of natural and ADCC cytotoxicity appear to be separate. (59 Refs)

70. ISOLATION AND CHARACTERIZATION OF THE Fc RECEPTORS OF THE MURINE LEUKEMIA L1210 (MEETING ABSTRACT).

Cooper SM, Sambray Y
USC Sch of Medicine, Los Angeles, Ca 90033
Fed Proc; 36(3):1486 1977

Cultured L1210 cells were labelled with ^{125}I by the lactoperoxidase technique and the plasma membranes prepared by partition of the cell lysates in a two phase system of polyethylene glycol and dextran. Glycoproteins (GP's) were extracted with lithium diiodosalicylate and passed over columns of Sepharose 4B coupled to either aggregated IgG or F(ab')2 fragments. 35.7% of the GP extract bound to the IgG column, whereas only 2.8% bound to the F(ab')2 column. Bound GP's were eluted with citrate buffer (pH 3.2) and analyzed by SDS-PAGE. Three components with approximate molecular weights of 65,000, 45,000, and 28,000 daltons were discovered. Previous results have shown that an Fc binding protein of 45,000 daltons is released from the cell surface after Fc receptor redistribution (J Immunol 117:511). The three components were purified by electroelution from SDS-polyacrylamide gels. The isolated molecules demonstrated the same affinity for different subclasses of mouse myeloma proteins as did the intact L1210 cells (IgG2a greater than IgG1 greater than IgG2b), although there were significant differences in the percentage of counts bound by individual myeloma proteins. Amino acid analyses of the two heavier components showed marked similarity. These results suggest that there may be molecular heterogeneity of Fc receptors on a single cell line. (Author Abstract)

271. ISOLATION OF A MURINE LEUKEMIA FC RECEPTOR BY SELECTIVE RELEASE INDUCED BY SURFACE REDISTRIBUTION.

Cooper SM, Sambray Y
Univ. Southern California Sch. Medicine, Los Angeles, CA 90033
J Immunol; 117(2):511-517 1976

The isolation of a membrane protein from the murine leukemia L1210, which has specific Fc-binding properties, is presented. The isolation technique involved surface cross-linking of the cell's Fc receptors with the use of aggregated human IgG and anti-human IgG. This resulted in the redistribution (patch formation and capping) of the cell's Fc receptors. Lactoperoxidase-catalyzed radioiodination of the cells, before complex binding, indicated that Fc receptor redistribution resulted in the selective release of surface proteins. SDS-PAGE analyses of the supernatants from cells thus treated revealed a major peak corresponding to a molecular wt of 45,000 daltons. This protein had been purified from the cell supernatants by immunoprecipitation and chromatography of the precipitates on Sephadex G-200 under dissociating conditions. After separation from the immune complex, this protein could be bound to heat-aggregated IgG, but not aggregated F(ab')2 fragments. The 45,000 dalton protein appeared to be the Fc receptor which was released from the cell surface in association with the complex.

272. CHARACTERIZATION OF AN FC RECEPTOR FROM A MURINE LEUKEMIA (MEETING ABSTRACT).

Cooper SM, Sambray Y
LAC USC Medical Center, Los Angeles, Ca 90033
Fed Proc; 35(3):857 1976

The murine leukemia, L1210, possesses an Fc receptor as determined by its ability to bind heat aggregated IgG and to form EA rosettes. If radiolabelled anti-human IgG is reacted with the bound aggregate, the complex becomes firmly attached to the cell's surface. Within one hour 30% of the complex is shed from the cell. This shedding is an active process related to patch formation and capping since it is markedly inhibited by 1 mM Na azide. SDS-PAGE of the complexes shed during the first hour of incubation indicates that the anti-human IgG has not been degraded. Lactoperoxidase catalyzed iodination of the cells reveals that some surface proteins are released along with the complex. Analyses of these supernatants by SDS-PAGE show a major peak of 45,000 daltons. Chromatography on Sephadex G-200, using non-dissociating conditions, indicates that the molecule is bound to a large molecular weight complex. This may represent the Fc receptor which has been released from the cell in association with the complex. (Author Abstract)

273. SEPARATE FC-RECEPTORS FOR IMMUNOGLOBULINS IGG2A AND IGG2B ON AN ESTABLISHED CELL LINE OF MOUSE MACROPHAGES.

Walker WS
St. Jude Children's Res. Hosp., Memphis, TN 38101
J Immunol; 116(4):911-914 1976

The specificity of Fc receptors on IC-21 cells, which were derived from mouse peritoneal macrophages with antibody-dependent effector cell activity, was examined. Only IgG2a and IgG2b myeloma proteins bound readily to IC-21 Fc receptors, the former in nonaggregated and aggregated form, the latter only as aggregated complexes. IgG2a bound in a manner characteristic of classically defined cytophilic antibody, while IgG2b binding appeared to be mediated by Fc receptors for antigen-antibody complexes. The results support the view that IC-21 macrophages possess separate and distinct Fc receptor sites for these two immunoglobulins.

274. FC-RECEPTORS ON MURINE T-CELL TUMORS AND ACTIVATED T-CELLS (MEETING ABSTRACT).

Krammer PH, Hudson L, Sprent J
Basel Inst. Immunology, Basel, Switzerland
Z Immunitaetsforsch Exp Klin Immunol; 150(3):216-217 1975

An indication that T cells might also carry Fc-receptors comes from studies of Fc-receptor positive T cell tumors in the mouse. Of 15 tumors, 11 expressed Fc-receptors on the surface of 5%-90% of the cells. Fc-receptors of all these tumors bound mouse IgG as well as rabbit IgG. Furthermore, in an EA rosette inhibition assay with purified myeloma proteins, the T tumor receptors were preferentially inhibited by IgG 2b. Using a rosetting system with ox RBC (ORBC) as indicator cells labeled with anti-ORBC antibody (IgG or IgM), various T cell populations were studied for the expression of Fc-receptors. Whereas IgM rosettes were not found, EA-rosettes were obtained with IgG labeled ORBC on in vivo mixed lymphocyte reaction activated T cells in the spleen. Activated T cells in the thoracic duct (TTDL) were Ig+ when prepared by injection of thymus cells into irradiated allogeneic hosts. The number of Ig+ cells in TTDL dropped markedly when B cell depleted thymus cells were used for preparation of TTDL. Both TTDL populations were Fc-receptor negative indicating that the Ig was derived from B cells in the thymus cell inocula for the preparation of TTDL and was probably bound by the free combining sites attaching to small particles of host antigen held by the antigen specific receptors of the TTDL.

275. RADIOLABELED ANTITUMOR ANTIBODIES. I. ANTIBODY-SPECIFIC AND IMMUNOGLOBULIN-SPECIFIC BINDING SITES ON MOLONEY LYMPHOMA CELLS (LSTRA).

Nord S, Weissman IL
Stanford Med. Sch., Calif.
J Natl Cancer Inst; 53(1):117-124 1974

A paired, radiolabeled immunoglobulin technique was used to show that LSTRA Moloney lymphoma cells may bind rat IgGa immunoglobulins at either antibody-specific (Fab) or immunoglobulin-specific (Fc) portions of the IgGa molecule. Fc-specific binding is inhibited in the presence of normal rat serum (NRS), whereas Fab-specific binding is not. Sodium dodecyl sulfate-polyacrylamide gel analysis of eluted radiolabeled anti-Moloney proteins (bound to LSTRA cells in the presence of NRS) revealed that the bound proteins were indeed immunoglobulin. The presence of an Fc receptor on a malignant lymphoma line was expected and may represent an important biologic marker for lymphoid cell subclasses.

276. MEMBRANE COMPONENTS ON A LYMPHOMA LINE (MEETING ABSTRACT).

Bergman Y, Blatt C, Haimovitch J
Dept. Chemical Immunology, Weizman Inst. Sci., Rehovot, Israel
Isr J Med Sci; 12(11):1360 1976

The membrane components of lymphatic leukemia cells, obtained from a line induced in SJL J mice by 7,12-dimethylbenzanthracene, were investigated. The intact leukemia cells lacked surface immunoglobulin (Ig) as measured by indirect immunofluorescence, but they possessed receptors for the Fc fragment as detected by the formation of EA rosettes. No secretion of Ig by the tumor cells was observed in the isoelectric focusing pattern of sera from tumor-bearing mice. Components of the cell membrane were radioiodinated by the lactoperoxidase technique and extracted with the nonionic detergent NP-40. The cell extract was then incubated with mouse Ig and goat antimouse Ig or with RNase and rabbit anti-RNase. The resulting precipitates were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate and mercaptoethanol. Both precipitates contained three major types of polypeptide chains with an apparent molecular wt of 45,000, 30,000, and 20,000. Smaller amounts of two polypeptide chains with a molecular wt of 13,000 and 11,000 were also observed. In sequential experiments, where non-specific precipitation was performed before specific precipitation, labeled proteins were not observed in the specific precipitate. It is concluded that the SJL J cell line produces plasma membrane protein(s) that bind to antibody-antigen precipitates. (no refs)

277. LYMPHOSARCOMA CELL GROWTH IS SELECTIVELY INHIBITED BY B LYMPHOCYTE MITOGENS: LPS, DEXTRAN SULFATE AND PPD.

Ralph P, Nakoinz I, Raschke WC
Salk Inst. Biol. Stud., San Diego, Cal.
Biochem Biophys Res Commun; 61(4):1268-1275 1974

A series of murine lymphosarcomas was adapted to culture. Cell growth was inhibited by B lymphocyte mitogens lipopolysaccharides (LPS), dextran sulfate, and purified protein derivative (PPD) a protein preparation from Bacilius Calmette Guerin (BCG), at concn which did not affect other hematopoietic cell lines. LPS inhibited the growth of RAW8 (a lymphosarcoma induced by Abelson leukemia virus) cells in culture after 2 d over the range from 0.1 to 100 ug/ml. Cell growth was blocked 50% at 0.2 ug/ml. The inhibition of RAW8 was reversible. RAW8 cells are also sensitive to dextran sulfate, 2 ug/ml inhibiting 50% growth and 100 ug being toxic. It is suggested that the cell lines described represent lymphocytes at different stages of maturation, and that they retain the pattern of mitogen interactions characteristics of that stage. The tumor lines in Class I arise from stem cell lymphocytes sensitive only to dextran sulfate. Cells in Class II are derived from early lymphocytes sensitive to PLS, and in Class III from late lymphocytes sensitive to PPD. These data should be of aid in defining the properties of normal B lymphocytes during differentiation, including the sequential appearance of surface antigens, receptors for immunoglobulin, and complement and mitogen sensitivity. The use of mitogens may also aid diagnosis of undifferentiated leukemias and lymphomas and allow classification of B lymphocyte neoplasm according to the stage of cell maturation, leading to improved therapy. (19 refs)

278. BIOSYNTHESIS AND SECRETION OF ANTIBODIES BY CHICKEN LYMPHOID CELLS - II. COOPERATION BETWEEN CARRIER-SPECIFIC T AND HAPTEN-SPECIFIC B-LYMPHOCYTES FOR THE DEVELOPMENT OF ANTI-HAPTEN IMMUNE RESPONSE.

Choi YS
Memorial Sloan-Kettering Cancer Center, Walker Lab., Rye, NY 10580
Immunochemistry; 14(1):59-62 1977

The biosynthetic assay method that can be used to study T and B lymphocyte cooperation in antibody response to trinitrophenyl human gammaglobulin is described. B histocompatible (B B) chickens of the SC line were used for all experiments. Since the immune response that developed in these chickens was entirely dependent on the number of B lymphocytes transferred, four daily injections of 4 mg cyclophosphamide at hatching made the chickens immunologically inert. The biosynthetic assay for specific antibody combined with adoptive transfer of immune lymphocytes could be used to study cellular cooperation between carrier-primed T lymphocytes from agammaglobulinemic chickens and hapten-immune B lymphocytes from normal chickens in the anti-hapten immune response. The studies suggest that the receptors for carrier proteins of T lymphocytes are

operationally distinct from the receptors for hapten of B lymphocytes. (25 refs)

279. MECHANISMS BY WHICH HAPTEN CONJUGATES OF PNEUMOCOCCAL POLYSACCHARIDE INTERFERE WITH THE CHALLENGE OF ANTI-HAPTEN MEMORY CELLS.

Romano TJ, Lerman SP, Thorbecke GJ
New York Univ. Sch. Medicine, New York, NY 10016
Eur J Immunol; 6(6):434-442 1976

Experiments were conducted on LAF1 mice to see if the removal of trinitrophenylated (TNP)-pneumococcal polysaccharide type 3 (SIII) would result in the recovery of memory function of B cells and to determine the nature of the receptor responsible for the binding of the TNP-SIII to the B cell surface. Incubation of TNP hemocyanin (TNP-KLH)-primed spleen cells with microgram amounts of 2,4-dinitrophenyl (DNP) or 2,4,6-trinitrophenyl conjugates of SIII for as little as 5 min at 4 C resulted in a specific "block" of the 19 S and 7 S adoptive memory response to TNP-KLH. This block appeared to be an example of specific receptor blockade. The block was specific and could be prevented by simultaneous incubation of the primed cells with hapten-protein conjugates which presumably competed with the hapten-polysaccharide for attachment to the B cell surface via anti-hapten Ig receptors. Removal via capping of these Ig receptors by exposure of TNP-KLH-primed memory cells to rabbit anti-mouse Fab serum for 45 min at 37 C rendered them refractory to the blocking effect of hapten-SIII. Once the hapten-SIII was attached to the memory cells, these blocked cells could be "rescued" through incubation with either mouse anti-SIII at 37 C or rabbit anti-DNP serum at 4 C. Since a papain digest of the IgG fraction of rabbit anti-DNP did not rescue the cells while the intact IgG did, a capping off of the TNP-SIII was proposed as the mechanism for this return to responsiveness of the hitherto blocked cells. A rescue was not seen by treatment of recipient mice with such B cell mitogens as dextran sulfate, endotoxin or purified protein derivative of tuberculin.

280. DIFFERENTIATED FUNCTIONS EXPRESSED BY CULTURED MOUSE LYMPHOMA CELLS. II. THETA ANTIGEN, SURFACE IMMUNOGLOBULIN AND A RECEPTOR FOR ANTIBODY ON CELLS OF A THYMOMA CELL LINE.

Harris AW, Bankhurst AD, Mason S, Warner NL
Hall Inst. Med. Res., Victoria, Australia
J Immunol; 110(2):431-438 1973

Nineteen different radiation-induced thymomas originating in BALB/c, NZB and NZW mouse strains were tested for Ig synthesis by short-term culture with 14C-amino acids followed by radioimmunoelectrophoresis. Twelve were negative, six produced light (L) chains and one synthesized a protein similar to IgM. The IgM producer, WEHI-22, and one of the L chain producers, WEHI-105, were established as cloned cultured cell lines and shown to possess the lymphoid cell characteristics of susceptibility to inhibition by low concentrations of thymidine and of cortisol. Both also possessed the theta antigen, a thymus-derived (T) lymphocyte marker. Cells of the WEHI-22 line also possessed surface immunoglobulin readily detectable by binding of labeled anti-Ig antibody and were able to bind mouse antibody-coated sheep erythrocytes forming rosettes, while WEHI-105 did neither. The specificity of the Ig receptor mediating rosette formation appeared from myeloma protein competition experiments to be very similar to that of the receptor described for non-thymus-derived (B) cells. It is considered that WEHI-22 cells either possess a mixture of T and B cell characteristics or are neoplastic variants of T cells possessing surface IgM with antiglobulin activity.

281. RETICULUM CELL SARCOMA: AN EFFECTOR CELL IN ANTIBODY-DEPENDENT CELL-MEDIATED IMMUNITY.

Ralph P, Prichard J, Cohn M
Salk Inst. Biol. Stud., San Diego, Calif.
J Immunol; 114(2):898-905 1975

A transplantable, murine reticulum cell sarcoma is described. Initially arising in a female BALB/c/NIH mouse, the tumor (J774) was ascitic. Experiments were performed with cells from passages 7-24 and included an erythrocyte antigen-binding assay and a chromium release assay for the antibody-directed cytoxicity. The J774 tumor was characterized by metastases to all abdominal organs and lungs and appeared microscopically identical to the type A reticulum cell sarcoma. It readily took up red stain and carbonyl iron. Specific binding of SRBC or burro RBC by J774 cells in the presence of appropriate mouse anti-erythrocyte sera was observed. The cells were also highly efficient in the lysis of 51Cr-labeled SRBC in the presence of rabbit or mouse anti-SRBC serum, releasing 50-77% of the radioactivity. The J774 cells were five times as active as spleen cells in lysing RBC in the presence of the appropriate antiserum. Special tests precluded the possibility that such antibody-dependent cytoxicity was due to contaminating normal peritoneal cells. With the aid of myeloma proteins, aggregated with bis-diazotized benzidine and labeled with 125I, it was found that the J774 cells bound IgG2a and IgG2b; to a lesser extent, the cells bound IgG1, but not IgM, IgG3, or IgA. Thus, the J774 cells appear to carry receptors for IgG2a and IgG2b. The reticulum cell sarcoma line thus has a number of properties characteristic of macrophages, including adherency, phagocyticity, specific binding of antibody-coated RBC and effector cell function in antibody-dependent cytotoxicity.

282. B AND T LYMPHOCYTES IN LYMPHOID TISSUES DETECTED BY MEANS OF CYTOPHOTOMETRY AND 51CR-LABELED MARKER ERYTHROCYTES: CLINICAL IMPLICATIONS (MEETING ABSTRACT).

Jonsson V, Christensen BE, Kofod B
Med. Dept. C, Hematology Div., Gentofte Univ. Hosp., Copenhagen, Denmark
Scand J Immunol; 6(6-7):713 1977

A new method for measuring the number of E-receptor-carrying T-lymphocytes and Fc on EAC-receptor-carrying B-lymphocytes in lymphoid tissue is described. Consecutive 6-micrometer cryostat sections of freshly extirpated lymphoid tissue were incubated in close chambers by the method of Tonder with the different marker RBC. The sections with adsorbed marker RBC were drop-stained with highly diluted eosin after fixation. The density of marker RBC was measured by cytophotometry at 530 nanometers (corresponding the eosin color) in a microphotometer. The total amounts of lymphocytes in the sections were calculated by comparing the total protein content of the sections with the total protein content of another part of the same tissue specimen of known wet wt and known B- and T-lymphocyte counts. The number of adsorbed marker RBC per lymphocyte was estimated by means of Cr51-labeled marker RBC and the radioactivity of the incubated sections was compared with the radioactivity of a standard suspension of marker RBC. A semiquantitative estimation of the number of receptors per lymphocyte is suggested. The diagnostic use and the use for estimating the effect of immunosuppressive treatment are discussed. A normal material is presented and compared with the B- and T-lymphocyte counts in peripheral blood of the same persons. Also, the results from 16 patients with malignant lymphocytic diseases, investigated before and during immunosuppressive treatment, are shown. (no Refs)

283. INTERACTION OF C-REACTIVE PROTEIN WITH LYMPHOCYTES AND MONOCYTES: COMPLEMENT-DEPENDENT ADHERENCE AND PHAGOCYTOSIS.

Mortensen RF, Osmand AP, Lint TF, Gewurz H
Dept. Biology, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI

J Immunol: 117(3):774-781 1976

The serum constituent C-reactive protein (CRP), which activates the classical complement (C) pathway when reacting with its substrates, was examined for its ability to mediate reactions of opsonic adherence and phagocytosis. The CRP was purified from human ascites or pleural fluids by affinity chromatography. RBC coated with C-polysaccharide (CPS) and reacted with CRP (E CPS-CRP) failed to adhere to B cells and displayed only minimal adherence to monocytes. However, upon the addition of absorbed C or purified C components these cells possessed the cleavage products C4b and C3b, which in attachment of these cells to both human B lymphocytes and peripheral blood monocytes. E CPS-CRP treated with C in the absence of antibody were readily phagocytosized by glass-adherent human monocytes. The phagocytosis of E CPS-CRP-C was not only mediated by CRP but also required the presence of CRP on the surface of the RBC. The extent of ingestion was proportional to the amount of CRP on the RBC intermediate and was reduced by blocking monocyte receptors with aggregated human gamma-globulin (HGG) at concentrations which did not impair the uptake of other particles. These results point to several analogous functional activities between CRP and antibody; in the latter case, such activities have usually been dependent on the Fc region of immunoglobulin. The mediation by CRP of reactions of opsonic adherence and phagocytosis as outlined in these studies points to a significant role for CRP in reactions of host defease and inflammation. (47 refs)

284. NONSPECIFIC SUPPRESSION OF T LYMPHOCYTE RESPONSES IN MICE CARRYING PROGRESSIVELY GROWING TUMORS.

Kilburn DG, Smith JB, Gorczynski RM
Dep. Zool., Univ. College London, England

Eur J Immunol: 4(12):784-788 1974

Unfractionated spleen cells from BALB c and C3H He mice with 4 different types of progressively growing tumors were unable to show enhanced DNA or protein synthesis upon stimulation with phytohemagglutinin (PHA) in vitro. The tumors included murine sarcoma virus- and polyoma virus-induced tumors, a transplanted spontaneous fibrosarcoma, and a transplanted ethylnitrosourea-induced trigeminal nerve tumor. After separation by velocity sedimentation, or passage over an anti-immunoglobulin column, cells were obtained which could respond to this mitogen. An auxiliary population of cells obtained by velocity sedimentation, sedimenting with the characteristics of activated B lymphocytes and sensitive to the cytotoxic effects of anti-immunoglobulin serum and complement, could depress the response of normal spleen cells challenged with PHA. A role for this cell type in the tumor progression seen in the host animals is discussed in terms of the effects of antigen-antibody complexes. Specific blockade of mouse lymphocytes during tumor progression might be due to complexes binding with antigen-specific receptors on the lymphocyte surface, while nonspecific blockage might be due to binding with lymphocytes by an Fc-type receptor.

285. DETECTION, PURIFICATION AND FUNCTIONAL CHARACTERIZATION OF A SIG NEGATIVE HUMAN LYMPHOCYTE SUBPOPULATION (MEETING ABSTRACT).

Papermaster VM, Denis KA, Harris NS
Dept of Surgery and Human Biological Chemistry and Genetics, Univ of Texas Medical Branch, Galveston, Tx 77550

Fed Proc: 36(3):1314 1977

An anti-human plasma cell serum (HuAPS) prepared against a cultured plasmacytoma cell line has been previously shown to recognize a specific antigen (PC) on human lymphocytes. The PC-bearing lymphocytes are B cells which have no C3 or SIg receptors; however, over 50% of this population appears to bear Fc receptors. Utilizing our monospecific antiserum we can isolate PC**+ cells by two methods: 1. a rosette method using SRBCs coated with APS via CrCl3 coupling, and 2. the lymphocyte separation method of Nash (1976) using APS attached to staphylococcal protein A-coated surfaces. The isolated PC**+ lymphocytes are examined for surface staining, E, EA and EAC rosetting, as well as tested in in vitro functional tests for their competence in: 1. PFC (Lymphocytes from human volunteers immunized with typhoid vaccine assayed in a modified Jerne hemolysis-ingel system against Salmonella lipopolysaccharide), 2. ADCC (antibody dependent cell mediated cytotoxicity using rabbit antibody-coated Chang liver cells as targets). Correlation of the data so obtained is expected to contribute to the identification of the effector cell responsible for ADCC; called the "K" cell, it is known to be of lymphoid as opposed to monocytoid lineage, to possess Fc but no SIg receptors; however, to date it cannot be positively identified. (Author Abstract)

286. CELL COLLABORATION BETWEEN T AND B LYMPHOCYTES AND MACROPHAGES IN ANTIBODY PRODUCTION IN VITRO. (PP. 1-26)

Feldmann M, Erb P, Kontiainen S

Lymphocytes and Their Interactions, Williams RC, ed. New York, Raven Press, 1975.

A study of T-B cell cooperation in their responses to hapten-protein conjugates was made to differentiate between two different mechanisms of receptor linkage (direct T-B contact with an antigen bridge or, with T cell receptors shed, possible receptor-antigen linkage on the membrane of another cell) and between other models of cooperation. Since most of the results were published elsewhere, only those more recent and less understood are reported. The authors describe four different functions, in which the central role of IgT in the induction and regulation of cooperative antibody responses is stressed. The first is induction of specific cooperation, whereby T cells release specific factors important in T cell suppression and antigenic competition. The second, specific T cell suppression, is initiated by the direct binding of IgT-antigen complexes to the surface of the lymphocytes. The third, antigenic competition, is caused by a blockage of macrophage surface receptor sites for IgT. In the fourth, Ig immunoglobulin purified from u-positive Ig-positive tumors blocks thymus-dependent IgM responses, but at low concentrations it augments IgG responses. It is not known if these functions are mediated by a single or by multiple classes of IgT. (58 refs)

287. RELEASE OF ANTIGEN-SPECIFIC IMMUNOGLOBULIN FROM CYTOTOXIC EFFECTOR CELLS AND SYNGENEIC TUMOUR IMMUNITY IN VITRO.

Rollinghoff M, Wagner H, Cone RE, Marchalon JJ
Royal Melbourne Hosp., Victoria, Australia

Nature New Biol: 243(122):21-23 1973

Cortisone -resistant thymus (T) cells were purified from cortisone-treated BALB c mice and were sensitized in vitro to

a tumor-associated transplantation antigen (TATA) of a syngeneic plasma tumor cell line (HPC)108. Following 125I labeling of the activated T cell surface proteins, the proteins were released from the cells by incubation in culture medium at 37 C and purified by dialysis. They were identified as IgM by polyacrylamide gel electrophoresis after precipitation with specific antisera. Twenty to 25% of the precipitated 125I labeled surface protein from activated T cells would bind to HPC 108 cells. This binding was eliminated if the immunoglobulin was removed by specific coprecipitation before the binding assay. Cell-surface immunoglobulin from non-activated T cells did not bind to HPC 108 cells. These results suggested that membrane bound immunoglobulin may function as the receptor for antigen on TATA activated T cells.

288. ANTIGEN BINDING TO LYMPHOID CELLS FROM UNIMMUNIZED MICE. II. HIGH FREQUENCY OF ANTIGEN-BINDING CELLS FOR SEVERAL PROTEIN ANTIGENS BY A MORPHOLOGICALLY DISTINCT IG-BEARING POPULATION OF T- AND B-LYMPHOCYTES.

DeLuca D, Miller A, Sercarz E
Dept. Bacteriol., Univ. California, Los Angeles, Calif. 90024

Cell Immunol; 18(2):255-273 1975

The binding of five protein antigens in prefixed spleen and bone marrow cells from unimmunized male and female A/J mice was studied by direct fluorescence and enzymatic assay. The mean antigen-binding cell frequency for each of the protein antigens (horseradish peroxidase, horse spleen ferritin, keyhole limpet hemocyanin, glucose oxidase, and beta-galactosidase) was close to 1% in the spleen and 3% in the marrow. LA subpopulation of immunoglobulin (Ig)-bearing cells in cell suspensions of bone marrow or spleen, in splenic T-cells, and in cortisone-resistant thymocytes could be defined by a characteristic nonuniform, speckled staining pattern with fluorochrome-labeled anti-Ig. This population contained most of the cells that bound enzymatically active or fluorochrome-labeled protein antigens. These antigens bound in the same characteristic pattern as did anti-Ig, and their binding was inhibited by a specific anti-immunoglobulin serum. The patterned antigen-binding subpopulation shed their receptors rapidly, making the use of fixed cells essential for the detection of the antigen-binding cells. Among the patterned antigen-binding cells, the frequency of cells binding a large, multideterminant antigen was very high: 30-50% of the cells bore Ig receptors for any of the five proteins. Patterned antigen-binding cells in splenic T-cell preparations and in cortisone-resistant thymocytes bore T-antigens as well as surface Ig.

289. AFFINITY ISOLATION OF A MACROPHAGE SURFACE MEMBRANE RECEPTOR FOR AGGREGATED IGG (MEETING ABSTRACT).

d'Urso-Cowgrd M, Cone RE
Yale Univ Sch of Medicine, New Haven, CT 06510

Fed Proc; 36(3):1253 1977

Detergent soluble membrane polypeptides with binding affinity for IgG were isolated from the P388D1 macrophage cell line by affinity chromatography with aggregated IgG covalently linked to Sepharose beads. SDS-PAGE analysis of radiolabeled surface membrane proteins which do not bind to Sepharose:IgG showed depletion of membrane proteins occurring predominantly in the 80,000d region. SDS-PAGE analysis of membrane polypeptides eluted from Sepharose:IgG by low pH or high salt concentration demonstrated a single 80,000d component which was not bound by Sepharose 4B or Sepharose:BSA columns using identical conditions. Cellular binding characteristics for molecular sieved fractions of aggregated IgG could be correlated with soluble receptor binding characteristics for these sieved IgG fractions coupled to Sepharose. The 80,000d protein does not readily aggregate, is resistant to degradation at 4 degrees C for 2 days, is not composed of disulfide linked subunits, and does not appear to contain much carbohydrate. During attempts to isolate receptors for IgG, certain characteristics of the Sepharose:IgG affinity columns employed were noted and will be discussed. (Author Abstract)

290. HISTAMINE RELEASE BY THE ANTIBODIES AGAINST RAT BASOPHILIC LEUKEMIA CELL MEMBRANE (MEETING ABSTRACT).

Ishizaka T, Chang TH, Taggart M, Ishizaka K
The Johns Hopkins Univ, Sch of Med, Baltimore, Md 21205

Fed Proc; 36(3):1217 1977

Attempts were made to prepare antibodies against IgE-receptors on mast cells. Rat basophilic leukemia cells (RBL) were saturated with rat E myeloma protein and their cell membrane was solubilized with Nonidet P40. Complexes composed of IgE and receptors were precipitated by anti-IgE, and a rabbit was immunized with the specific precipitates included in complete Freund's adjuvant. Antibodies against cell membrane were specifically purified using RBL as an immunosorbent, and then absorbed with mast cell-depleted rat peritoneal cells. No anti-IgE was detected in the final preparation. The antibodies specifically bound with RBL and normal rat mast cells, as demonstrated by indirect immunofluorescence. The anti-RBL membrane (anti-RBL) quantitatively inhibited the binding of ^{125}I labeled IgE with RBL or normal rat mast cells. Saturation of IgE-receptors on RBL with rat IgE partially inhibited the binding of rabbit antibodies with the cells, indicating that a significant proportion of anti-RBL is anti-receptor. Anti-RBL antibody gave an immediate skin reaction in normal rats at 1:3200 to 1:6400 dilution. The antibodies induced histamine release from peritoneal mast cells of Hooded Lister inbred rats. (Author Abstract)

291. BINDING OF IGM-COATED ERYTHROCYTES TO CHRONIC LYMPHATIC LEUKEMIA (CLL) CELLS (MEETING ABSTRACT).

Pichler WJ, Knapp W
Inst. Immunology, Vienna, Austria

Scand J Immunol; 6(6-7):736 1977

Bovine RBC coated with immunoglobulin (Ig) M antibodies (EA-IgM) were found to bind to lymphocytes (0%-78%; mean, 17.6%) in 25 chronic lymphatic leukemia (CLL) patients. The absence of IgG in our anti-bovine RBC IgM preparation was evaluated with protein A and in a macrophage system. In contrast to the EA-IgM binding to a subpopulation of peripheral T lymphocytes in normal individuals, binding of EA-IgM to lymphocytes of CLL patients occurred also without prior incubation of lymphocytes, although the rosette values with CLL cells increased after incubation for 16 hr at 37 C (mean, 21.5% in comparison with 17.6%). By mixed rosette techniques it was found that in CLL patients EA-IgM bind to those cells which bear receptors for EA-IgG as well, but not to the sheep RBC receptor-bearing lymphocytes. Absorption of the anti-bovine RBC antiserum with CLL cells in the cold did not alter the binding of EA-IgM. The rosette formation of CLL cells with EA-IgM could be inhibited by addition of normal rabbit or human serum, by purified human IgM, but not by aggregated human IgG or by fetal calf serum. (no Refs)

292. IMMUNOGLOBULINS AND COMPLEMENT RECEPTORS ON CLL CELLS.

Grey HM, Kubo RT, Rabellino EM, Polley M, Ross GD
Natl. Jewish Hosp. and Res. Center, Denver, CO 80206
Adv Biosci; 12:213-224 1974

Data are presented to support the hypothesis that the predominance of IgM on the surface of chronic lymphatic leukemia (CLL) does not represent a situation found in normal peripheral blood lymphocytes but may be a result of neoplastic transformation or lack of differentiation, or a dedifferentiation, on the part of CLL cells. Two test systems were used to evaluate the relative amounts of IgG- and IgM-bearing cells in normal human peripheral blood: one is an inhibition system using radiolabeled Ig antigens and specific anti-Ig reagents, and the second is a surface iodination and immunoprecipitation procedure using lactoperoxidase to label the lymphocyte surface proteins enzymatically. Both procedures indicated that, in normal human peripheral blood, there is twice as much IgG as IgM associated with the lymphocyte population. The preponderance of the IgM class in CLL does not appear, therefore, representative of normal peripheral blood. The quantitative amounts of Ig on CLL cells also appear to be quite different from what is found on normal lymphocytes. Earlier studies of Ig's associated with CLL cells indicated that about 20% of the cases showed either no Ig or only light chains on the lymphocyte surface. Immunofluorescence with anti-IgD antisera, however, indicated that these were IgD leukemia. These preliminary data suggest that a relatively high incidence of IgD leukemias would be encountered in CLL. In 15/18 CLL cases investigated for complement receptor, leukemic cells reacted preferentially with mouse complement compared to human complement. In six, little or no reactivity with human complement was observed. Further studies indicated that there are two receptor sites for C3 present on normal lymphocytes: one recognized C3b, the only form of C3 on the EAC human; the second recognized a split product of C3b (probably C3d). This latter receptor site would recognize the EAC mouse since both C3b and C3d would be expected to be present on these sensitized erythrocytes. This indicates that in CLL there is a strong tendency for the C3b receptor to be deficient or absent, so that EAC mouse reacts preferentially with these cells.

293. ANOMALOUS CAPPING BEHAVIOR OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) LYMPHOCYTES DESPITE NORMAL ANTIBODY BINDING AND TUBULIN LEVELS. (MEETING ABSTRACT).

Liebes L, Fleit H, Ambady S, Quaglata F, Silber R
N Y U Sch Med, New York, NY 10016
Fed Proc; 36(3):711 1977

The binding of multivalent ligands to receptors on lymphocyte surfaces causes the redistribution of receptor-ligand complexes to polar caps demonstrable by fluorescent microscopy. Capping requires energy and may involve microtubules and actomyosin-rich microfilaments. The percentage of capped cells is much lower when CLL than when normal blood lymphocytes are exposed to anti-IgG antiserum. This difference in cell surface dynamics was investigated as follows: Antilymphocyte sera (ALS) were raised in rabbits injected with CLL lymphocytes. Although these antisera were directed against CLL cells and agglutinated normal and CLL lymphocytes with the same titer, decreased capping was observed with CLL lymphocytes: Using the indirect immunofluorescent technique, 39% of normal and 14% of CLL lymphocytes were capped (p less than 0.001) by the ALS. The amount of ligand bound to the cells was quantitated with ^{125}I-labelled ALS IgG. Similar amounts of ALS IgG, 2 x 10^{6} molecules/cell, were bound by normal blood, tonsil or CLL lymphocytes. The concentration of tubulin in normal blood lymphocytes was 2.63 plus or minus 0.77 mg/g protein which was the same as that of CLL lymphocytes (2.74 plus or minus 1.1 mg/g protein). These results indicate that CLL lymphocytes manifest an impaired receptor distribution despite the binding of equivalent amounts of ligand and the presence of normal tubulin levels. Possible differences in other contractile proteins and energy dependent reactions need to be investigated. (Author Abstract)

294. ASSAY AND SOME PROPERTIES OF SOLUBILIZED CELL RECEPTOR FOR IGE (MEETING ABSTRACT).

Newman SA, Rossi G, Metzger H
NIH Bethesda, Md 20014
Fed Proc; 35(3):808 1976

Rat IgE which is bound to the non-ionic detergent solubilized cell receptor for IgE (from rat basophilic leukemia cells) is precipitated at concentrations of (NH4)2(SO4) where free IgE is soluble. This finding has permitted us to assay for functional receptor rapidly and accurately. Under conditions where approximately equal to 65% of receptor-bound ^{125}I-IgE is precipitated (37% saturated (NH4)2(SO4)) only 4% of free IgE is sedimented. Saturation of the receptor by IgE can be demonstrated and a linear response to the amount of receptor added is obtained. Using this assay 1) It can be shown that the receptor in the crude cell lysate is highly unstable at 37 degrees C (1/2 time of functional decay approximately equal to 10 min) and even at room temperature. Considerable protection occurs if the receptor is saturated with IgE. 2)Binding kinetics (at 4 degrees C) show k1 approximately equal to 5 x 10^{7} M**-1 sec**-1, 25x greater than the rate constant observed with intact cells at this temperature k1 less than or equal to 5 x 10**-6 sec**-1 so that K(subA) greater than or equal to 1 x 10^{11} M**-1. 3) When the lysate is fractionated on Sepharose 6B, and the eluate assayed, functional receptor elutes at a position expected for a protein of 2.6 x 10^{5} mw. Under the same conditions the IgE-receptor complex has an apparent mw of 5.8 x 10^{6}. These data suggest that the solubilized receptor for IgE is monovalent. (Author Abstract)

295. CARRIER DETERMINED TOLERANCE WITH VARIOUS SUBCLASSES OF MURINE MYELOMA IGG.

Borel Y, Golan DT, Kilham L, Borel H
New England Medical Center Hosp., Tufts Univ. Sch. Medicine, Boston, MA 02111
J Immunol; 116(3):854-858 1976

Groups of BALB/c mice were treated with various conjugates of 2,4-dinitrophenyl (DNP) and BALB/c myeloma proteins belonging to the four subclasses of IgG (IgG1, IgG2a, IgG2b, IgG3). Immediately thereafter, they were challenged with DNP-keyhole limpet hemocyanin in complete Freund's adjuvant, and antibody to the hapten was measured by direct and indirect hemolytic plaque assay. All subclasses of IgG were effective as tolerance-inducing carriers. However, the ability to induce tolerance was shown to be dependent upon the concentration of hapten bound to each myeloma protein. Tolerogenic conjugates suppressed both direct and indirect plaque-forming cells in all types of antibodies measured (IgG1, IgG2a, IgG2b, IgGa), whereas, non-tolerogenic conjugate failed to suppress them. The intact molecule of IgG, but not its fragments (Fab, F(ab)2 ', Fc), appeared necessary as tolerance-inducing carriers. The ability to induce tolerance may be related to the capacity of the tolerogenic conjugates to cause receptor blockade.

296. IDIOTYPIC ANALYSIS OF LYMPHOCYTES IN VITRO. I. SPECIFICITY AND HETEROGENEITY OF B AND T LYMPHOCYTES REACTIVE WITH ANTI-IDIOTYPIC ANTIBODY.

Black SJ, Hammerling GJ, Berek C, Rajewsky K, Eichmann K

Inst. Genetics, Univ. Cologne, Cologne, W. Germany

J Exp Med; 143(4):846-860 1976

An idiotypic system which employs a myeloma protein instead of an induced antibody for the production of anti-Id was studied. Guinea pig anti-idiotypic antibodies (anti-Id) of the IgG1 class, directed to an A/J mouse antibody to Group A streptococcal carbohydrate (A-CHO), or directed to a BALB/c mouse myeloma protein that binds the same antigen, stimulated B-precursor cells as well as T-helper cells when injected into mice of the appropriate strain. The strain-specific induction of both precursor and helper activity was detected by in vitro secondary responses of primed spleen cells to A-CHO or to 2,4,6-trinitrophenyl (TNP) upon challenge with Group A streptococcal vaccine (Strep.A) or with TNP-Strep. A, respectively. B- and T-cell populations primed with anti-Id were uniform with respect to the binding of antigen and of anti-Id. This was in contrast to cells primed with Strep. A, which were heterogeneous. Taken together, B and T cells that possess the same antigen-binding specificity shared idiotypic determinants, revealed the same idiotypic polymorphism, and occasionally displayed similar degrees of heterogeneity with respect to the binding of antigen and anti-Id. Since the anti-Id used to detect Id determinants was associated with the heavy chain of the variable region of mouse antibodies, the data suggest that this region of the immunoglobulin molecule is shared between T- and B-cell antigen receptors. These data also confirm previous reports that anti-Id can presensitize an animal for a secondary response to the antigen which is complementary to the corresponding Id.

297. TRIGGERING OF HUMAN PERIPHERAL BLOOD B CELLS: POLYCLONAL INDUCTION AND MODULATION OF AN IN VITRO PFC RESPONSE.

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Div. Tumor Immunology, Sidney Farber Cancer Center, Boston, MA 02115

J Immunol; 115(5,pt2):2021-2028 1976

A system for the in vitro production of specific antibody by human peripheral B cells and the analysis of this response utilizing a modification of the Jerne plaque assay is described. Polyclonal activators were used to analyze the signals required to induce antibody synthesis by subpopulations of human peripheral blood lymphocytes. The in vitro synthesis of antibody directed against sheep RBC was measured by a modification of the Jerne plaque assay. The plaques contained central lymphoid cells, could be inhibited by anti-human immunoglobulin (Ig), and were strictly dependent on both the addition of complement and viable B lymphocytes actively synthesizing protein. Kinetic analysis showed that the plaque-forming cell (PFC) response developed over time during cell culture: peak activity occurred between days 6 and 7. Human peripheral blood B lymphocytes, isolated by Sephadex G-200 anti-Fab column chromatography, were nonspecifically induced by either pokeweed mitogen (PWM) or soluble products of antigen-activated human T cells to differentiate into PFC. In contrast, unfractionated peripheral blood lymphocytes, Ig- cells isolated from immunoabsorbant columns, and B cells enriched by nylon wool adherence were not triggered to antibody synthesis even in the presence of polyclonal activators. B cells isolated by nylon wool adherence and further fractionated by binding and elution from anti-Fab columns were then readily triggered by PWM to differentiate into PFC. These results suggest that the interaction of human B cells with anti-Fab columns, presumably at the Ig receptor, serves as an important signal in the differentiation events leading to PFC activity. The anti-Fab interaction alone, although critical, was not sufficient to induce the differentiation of precursor cells into PFC since subsequent triggering by antigen-activated T cells supernatants or PWM was required for the development of an optimal PFC response. Other data demonstrated that the PFC response could be regulated by autologous peripheral Ig- lymphoid cells in vitro. At low concentrations of PWM, Ig- cells consistently augmented the B cell PFC response whereas, at high concentrations of PWM, suppression was observed. Depletion of E rosetting cells from the Ig- population eliminated the subset responsible for augmentation but did not eliminate the suppressor cell activity. (42 refs)

298. EFFECTS OF LEVAMISOLE ON HUMAN MONOCYTE FUNCTION AND IMMUNOPROTEIN RECEPTORS.

Schmidt ME, Douglas SD

Department of Medicine, University of Marburg, Marburg, West Germany

Clin Immunol Immunopathol; 6(3):299-305 1976

Study of the effects of the antihelminthic drug levamisole on human monocyte function in vitro reveals that this drug enhances functional capacities. Monocytes demonstrate increased cytoplasmic spreading after incubation with levamisole for 2 hr. Levamisole increases chemotaxis, phagocytosis of bacteria, and activity of plasma membrane receptors for immunoglobulin IgG and complement C3. These observations suggest that levamisole may have potential use as an immunopotentiating agent in man. (Author Abstract)

299. THE CELL SURFACE-MEMBRANE COMPLEX IN CELL INTERACTIONS.

Edleman GM, Wang JL

Cell Surfaces and Malignancy John E Fogarty International Center for Advanced Study in the Health Sciences and NIH; Mora, P. T., ed. Washington, D.C.: US Dept. Health, Education, and Welfare Publication No. (NIH) 75-796; Fogarty International Center Proceedings No. 24, pp. 83-116; 1975

Cell-cell interactions and cell surface signaling depend on the physicochemical interactions of receptors, the specificity of these receptors, and their linkage to metabolic events within the cell. Experiments on patching and capping of lymphocyte surface receptors and the modulation of receptor and cell movements by lectins such as concanavalin A (Con A) and various drugs suggest that cell surface receptors may have a common anchorage system under the plasma membrane. It is proposed that this system involves reversible and cooperative interactions in an assembly consisting of receptors, microfilaments, microtubules, and their subunits. The structural homology of b2 -microglobulin (cell surface protein) with immunoglobulins and the physical association of this protein with histocompatibility antigens suggest the possibility that specific cell-cell recognition in development may be mediated by a diverse and degenerate set of receptors. Analyses of the kinetics of lymphocyte response to stimulation by Con A suggest that resting cells not only have a diverse set of receptors for cell-cell interactions, but also exist in a temporally dispersed and statistically distributed set of responsive states in the G1 portion of the cell cycle. This set of temporally dispersed states may themselves form a cycle. Although the modulation, diversity, and temporal dispersion hypotheses are derived largely from lymphocyte studies, they may apply to other differentiated cells as well.

C. Occurrence of Complement Receptors

300. CHARACTERISTICS OF THE EFFECTOR CELLS MEDIATING CYTOTOXICITY AGAINST ANTIBODY-COATED TARGET CELLS. II. THE MOUSE NONADHERENT K CELL.

Greenberg AH, Shen L, Walker L, Arnaiz-Villena A, Roitt IM
Middlesex Hosp., London, England
Eur J Immunol; 5(7):474-480 1975

An attempt was made to clarify apparent species differences in nonphagocytic effector cells of mice and humans in that such cells in mice are found in an adherent population of cells while such cells in humans are found in nonadherent populations as well. These particular effector cells were detected by their cytotoxicity for antibody-coated chicken red blood cells (CRBC). Spleen cell preparations from nonimmune BALB/c or (BALB/c x C57BL/6)F1 mice were treated with a combination of carbonyl iron and passage through glass beads to yield a fraction which contained less than 1% of monocytes and polymorphonuclear leukocytes, was incapable of phagocytosing SRBC-antibody-complement complexes, and contained reduced proportions of immunoglobulin-bearing cells. Cytotoxicity assays were carried out with rabbit antibody-coated CRBC as the target cells, where the antibody used was prepared against CRBC or consisted of Fab of rabbit anti-CRBC antibody coupled with dinitrobenzene sulfonic acid . The results showed that cells present in the nonadherent spleen cell preparation were capable of killing antibody-coated CRBC. This nonphagocytic effector cell, referred to as the nonadherent K (killer) cell, is distinguished from the previously described nonphagocytic myeloid K cell by its relatively weak surface adherence properties and low concentration within the mouse spleen. The ability of the nonadherent K cells to kill CRBC in the presence of rabbit antiserum differed from that of whole spleen cell preparations in that over 15 times higher concentrations of antiserum were required before optimal killing was reached, and significant cytotoxicity could be demonstrated with whole spleen cells at low antiserum concentrations where lymphoid preparations were virtually inactive. The nonadherent K cell was further characterized by its relatively large size, lack of theta or immunoglobulin determinants, the presence of Mg++-independent complement receptors, affinity for aggregated IgG2 myeloma proteins , inhibition by cytochalasin B and good survival in cell culture. The results indicate that the species differences are quantitative rather than qualitative, since it could be demonstrated that nonadherent lymphoid effector cells exist in mice as well as in humans.

301. BLOOD LYMPHOCYTES AFTER RADIATION THERAPY OF CARCINOMA OF PROSTATE AND URINARY BLADDER.

Blomgren H, Wasserman J, Littbrand B
Karolinska Inst., Stockholm, Sweden
Acta Radiol Ther (Stockh); 13(4):357-367 1974

Peripheral blood lymphocytes were studied from 15 men and 4 women pts aged 52-75 yr following external beam radiation therapy for prostatic or urinary bladder carcinoma. Radiation sources were either a ^{60}Co unit or a 6 MeV linear accelerator delivering total tumor doses of 3600 rad in 4 wk to 8400 rad in 28 wk for bladder and 5400 rad in 6 wk for prostatic carcinoma. Total peripheral blood lymphocytes were decreased from 1802+-266/ul before therapy to 788+-159/ul within 3 wk following completion of the course of radiation (56% decrease). The frequency of lymphocytes bearing membrane bound receptors to activated complement (i.e, thymus-independent cells) was significantly decreased (from 46.4+-6.9/ul to 24.3+-3.6/ul) whereas the frequency of lymphocytes agglutinating with sheep erythrocytes (i.e, thymus-dependent cells) was essentially unchanged (36.3+-7.3/ul vs. 42.6+-4.1/ul). The relative response of lymphocytes to phytohemagglutinin (PHA) was unchanged but their stimulation by purified proteins derivative (PPD) was significantly impaired following irradiation. This reactive lymphopenia is similar to that observed in breast carcinoma pts treated by radiation and cytostatic drugs. (22 refs)

302. EFFECT OF RADIOTHERAPY ON BLOOD LYMPHOCYTE POPULATION IN MAMMARY CARCINOMA.

Blomgren H, Berg R, Wasserman J, Glas U
Radiumhemmet, Karolinska Sjukhuset, S-104 01 Stockholm, Sweden
Int J Radiat Oncol Biol Phys; 1(3/4):177-188 1976

An analysis of the peripheral blood lymphocyte population in patients undergoing local radiotherapy for breast cancer is presented. Cellular composition and in vitro responsiveness to nonspecific mitogens and to purified protein derivative of tuberculin (PPD) were examined in three treatment groups: group A, local radiation followed in 6 wk by radical mastectomy; group B, radical mastectomy followed in 5-6 wk by local radiotherapy; and group C, radical mastectomy alone. The radiation target dose was 4,500 rads in 6-7 wk. A reduction in the number of peripheral lymphocytes of approx 50% accompanied pre- or postoperative irradiation. The B-cell population, as determined by the presence of membrane-associated receptors for activated complement, was significantly depressed (p less than 0.0005 for group A patients, and p less than 0.001 for B patients), while the T-cell population, as characterized by their ability to bind sheep RBC to their membrane, was increased (p less than 0.1 for group A and p less than 0.02 for group B). In both group A and group B patients, the in vitro response of lymphocytes to PPD was significantly reduced, but there was no significant change in PPD responsiveness in the lymphocytes of group C patients. The in vitro mitogenic response to phytohemagglutinin and to pokeweed mitogen was unaffected in all three groups. In none of the three groups was the antibody titer against Herpes simplex, Cytomegalo virus, or Morbilli affected. (39 refs)

303. LYMPHOCYTE ABNORMALITIES IN UNTREATED PATIENTS WITH HODGKIN'S DISEASE.

Holm G, Mellstedt H, Bjorkholm MB, Johansson B, Killander D, Sundblad R, Soderberg G
Department of Medicine, Seraphimer Hospital, P.O. Box 12700,S-112 83 Stockholm, Sweden
Cancer; 37(2):751-762 1976

The study comprises 38 unselected and untreated patients with Hodgkin's disease (HD) and 23 healthy persons. Highly purified blood lymphocytes were analyzed for cells forming rosettes with sheep red blood cells (T lymphocytes), and lymphocytes bearing surface immunoglobulin (B lymphocytes) and/or carrying receptors fo complement. Their DNA synthesis, spontaneously, or after activation with mitogens (phytohemagglutinin, concanavalin A, poke weed mitogen) and purified protein derivative (PPD) was measured. Delayed skin hypersensitivity to PPD and mumps antigen was studied. Most HD patients had low numbers of T lymphocytes (50% of the cases below normal range) while the mean B-lymphocyte level was normal but with a greater variation than in the control group. Lymphocytes from most patients were poorly stimulated by T-cell mitogens. Two-thirds of the patients and one healthy control had negative skin reaction to 2 TU PPD and the DNA synthesis of their lymphocytes after activation with PPD was low. Large lymphoid cells (greater than 9-mm diameter) were commonly present in Hd blood and the spontaneous DNA synthesis was high, particularly in lym-

phocytes from stage B patients. The percentage of T lymphocytes and the stimulation of lymphocytes by T-cell mitogens or by PPD, a T-lymphocyte function, did not correlate, and each test only detected defects in about half the cases. Simultaneous application of all tests revealed abnormalities of blood T lymphocytes in 33 out of 38 patients. Although the defects were usually more pronounced in patients with advanced disease, the impairment of T lymphocytes and their functions is present in all stages of Hodgkin's disease.

304. CORRELATION OF ADENOSINE DEAMINASE ACTIVITY AND CELL SURFACE MARKERS IN LEUKEMIC LYMPHOBLASTS. (MEETING ABSTRACT).

Smythe J, Poplack DG, Holiman BJ, Leventhal BG, Magrath IT

Pediatric Oncology Branch, Natl Cancer Inst, Bethesda, Md. 20014

Proc Am Assoc Cancer Res; 18:214 1977

Recently, adenosine deaminase (ADA) levels in blast cells of patients with acute leukemia were found to be elevated when compared to levels in normal lymphocytes. This observation prompted us to study ADA levels in acute lymphoblastic leukemia (ALL) and to investigate the possibility of a correlation between ADA activity and lymphoblasts differentiated on the basis of their cell surface markers. Lymphoblasts from previously untreated patients with ALL were evaluated for the presence of sheep erythrocyte and complement receptors by standard E and EAC rosetting techniques. In addition, sonicated extracts of these Ficoll Hypaque separated lymphoblasts were assayed spectrophotometrically for ADA activity. Of the 23 patients tested, 9 had T lymphoblasts (E rosette positive); 14 had null lymphoblasts (E and EAC rosette negative). Mean ADA activity was 3 times higher in T lymphoblasts (mean deltaO.D./min./mg protein=1.43) than in null lymphoblasts (mean deltaO.D./min./mg protein =.48). Thus, a significant (less than .02) positive correlation exists between elevated ADA levels and T cell ALL. These findings appear to offer a biochemical method of distinguishing between immunological sub-types of lymphoblasts which potentially is of prognostic and therapeutic value. (Author Abstract)

305. THE RAJI CELL RADIOIMMUNE ASSAY FOR DETECTING IMMUNE COMPLEXES IN HUMAN SERA.

Theofilopoulos AN, Wilson CB, Dixon FJ

Scripps Clinic and Res. Foundation, La Jolla, Calif. 92037

J Clin Invest; 57(1):169-182 1976

A sensitive and simple procedure for the detection and quantitation of soluble complement (C)-fixing immune complexes in sera of patients with various diseases utilizes C receptors on Raji cells. These cells lack membrane-bound immunoglobulin (Ig) but have receptors for IgG, Fc, C3b, C3d, and possibly other C proteins. Uptake experiments showed that both aggregated human gamma globulin (AHG) and 7S IgG bound to receptors for IgG Fc; however, AHG reacted with C bound to cells only via receptors for C and this binding was much more efficient than via IgG Fc receptors. AHG was used as an in vitro model of human immune complexes and its uptake by Raji cells was quantitated by ^{125}I-radiolabeled antihuman IgG. The limit of sensitivity of this test was 6 microg/ml serum. The ability of Raji cells to AHG in serum depended on the amount of radioactive antibody used and the size of aggregates. The presence of an excess of C somewhat inhibited binding of C-containing AHG to Raji cells . For assays on human sera, incubation mixtures of Raji cells and sera were reacted with excess radiolabeled antihuman IgG; the amount of radioactivity bound to the washed cells was determined and referred to a standard curve of radioactive antibody uptake by cells previously incubated with increasing amounts of serum AHG. Immune complexes were thus detected and quantitated in serum hepatitis, systemic lupus erythematosus, vasculitis, subacute sclerosing panencephalitis, dengue hemorrhagic fever, and more than 1/3 of 104 patients with malignancies including solid tumors, lymphoid tumors, and melanoma. The presence of the complexes in cancer patients suggests that tumor antigen-antibody complexes may circulate and deposit in tissues of these patients.

D. Carbohydrate Binding Proteins

306. BIOCHEMICAL STUDIES ON CELL ASSOCIATION AND ITS RELEVANCE TO NEOPLASIA.

Humphreys T

Cellular Membranes and Tumor Cell Behavior Annual Symposium on Fundamental Cancer Research, 1975; (Baltimore, Williams and Wilkins Co):173-192 1975

Biochemical studies of cell aggregation-enhancing factors in marine sponges, chicken and mouse embryos, mouse teratoma cells, and slime mold cultures are reviewed. An aggregation factor was isolated from the tissues of many sponge species by dissociation in cold calcium and magnesium-free seawater. Experiments involving the aggregation of cells from two different species in a mixed suspension indicated that the reaction of factor with cell surface is species specific. The factor from one sponge species has been purified and characterized as a large, negatively charged proteoglycan complex of 20 million daltons. Electron microscopy indicated that the factor consisted of fibers arranged as a central circle with arms branching off. Aggregation studies with chick and mouse embryos suggest that a receptor in the cell surface binds a specific polysaccharide in the enhancing factor. The enhancing factor protein then projects from the cell to bind the ligator, which is at least divalent and able to bind two cells together. It is further suggested that the ligator is analogous to the sponge aggregation factor and may be a related kind of molecule. Aggregation of trypsin-dissociated mouse teratoma cells growing as an ascites tumor of embryoid bodies proceeded much more rapidly in the presence of the ascites fluid, suggesting that the fluid contains a factor that is actually the molecule being synthesized from the polysaccharide precursors (glutamine or glucosamine) on which aggregation is dependent. The factor appears to be a ligand; it is specific; and partial characterization indicates that it is a large molecule containing protein and polysaccharide. Factor molecules are much less tightly bound to the tumor cells and tend to be lost rapidly, which would explain the high concentration of aggregation factor in the ascites fluid. As the molecular systems for cellular association are elucidated, it should be possible to determine the molecular basis for the change in a cell that causes it to leave its normal associations and become malignant.

307. THREE-DIMENSIONAL STRUCTURE OF L-ARABINOSE-BINDING PROTEIN. (MEETING ABSTRACT).

Quiocho FA, Phillips GN, Gilliland GL, Newcomer ME

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Fed Proc; 36(3):667 1977

The three-dimensional structure of L-arabinose-binding protein, an essential component of shock-sensitive active transport in E coli, has been determined at 3.5 A resolution and a preliminary 2.8 A map calculated. These analyses show that the protein is ellipsoidal (axial ratio=2:1). The molecule consists of 2 globular domains (P and Q domains). The first two-thirds of the polypeptide chain make up the 2 domains and the remainder provides most of the structural linkage between the 2 domains. The interior of each domain contains beta-pleated sheet structure and both beta-sheets are twisted

or "fanned" by about 90 degrees. The exterior of each domain contains 4 alpha-helices. The axes of all helices (9 of more than 2 turns) are roughly parallel to the long axis of the ellipsoidal molecule. Likewise, the chains comprising the beta-sheet structures are more or less oriented along the long axis of the molecule. Between the 2 domains is a cleft. The single essential cysteine residue is located in this cleft, near the P domain. (Author Abstract)

308. IMMOBILIZED CARBOHYDRATES AS CELL SURFACE ANALOGUES: A MODEL SYSTEM FOR CELL-CELL INTERACTIONS.

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Diss Abstr Int B; 38(1):37-B 1977

Aminohexyl glycosides were immobilized on Sephadex G-25 beads using the cyanogen bromide procedure. Cells grown in the presence of P32 phosphate or C14 glucose readily transferred label preferentially to galactose beads. The transferred label was not released from beads by a variety of detergent, buffer or acid washes. The reproducible transfer of radiolabel from SV 40/3T3 cells preferentially to galactoside beads was obtained. The P32 label was identified as RNA. Furthermore, RNA from lysed cells (or even purified, protein-free RNA) bound preferentially to the galactoside beads. The observed sugar specific transfer of RNA may be due to a small amount of cell lysis, followed by preferential binding of released RNA by galactoside beads. The chemical basis for the differential binding is not understood. However, the possibility that it is due to an unidentified side product of the CNBr coupling procedure has led us to develop another system for carbohydrate immobilization. Active esters of acrylic acid (N-succinimidyl acrylate, N-phthalimidyl acrylate, and p-nitrophenyl acrylate) were synthesized, then copolymerized with acrylamide and N,N'-methylenebisacrylamide. Displacement of the active ester in the gel by various ligands containing amino groups resulted in the formation of stable amide bonds between the ligands and the polyacrylamide gel. This gel was resistant to chemical and microbiological degradation, and ligand levels and porosity were easily controlled. This immobilization method was also used to study the interaction of cells with immobilized carbohydrates. Gel discs were activated by copolymerizing active ester acrylate at their surfaces, and eight different 6-aminohexyl D-pyranosides were incorporated as amides. Six different cell types were tested for their ability to adhere to the derivatized surfaces. Chicken hepatocytes showed sugar specific gel adhesion. Seventy to 100% of juvenile chicken liver cells in suspension bind to N-acetyl-glucosamine (GlcNAc) gels within 60 min at 37 C, while less than 5% of the cells adhere to any other sugar-containing or control gels. The cells adhere avidly to GlcNAc gels, resisting dissociation by repeated washing. Cell-gel binding requires Ca2+, and is not inhibited by inhibitors of oxidative phosphorylation or low temperature. It is inhibited or reversed by free GlcNAc or N-acetylglucosaminides. The most effective inhibitor used was asialo-agalacto-orosomucoid. Although the binding activity shares many properties with a chicken hepatocyte binding protein described by Lunney and Ashwell, its identity and physiological role have not been determined. (Author abstract) (no Refs)

309. DEVELOPMENTALLY REGULATED PROTEINS OF THE PLASMA MEMBRANE OF DICTYOSTELIUM DISCOIDEUM. THE CARBOHYDRATE-BINDING PROTEIN.

Siu CH, Lerner RA, Ma G, Firtel RA, Loomis WF
Scripps Clinic and Res. Foundation, La Jolla, CA 92037
J Mol Biol; 100(2):157-178 1976

Proteins of the plasma membrane of Dictyostelium discoideum were characterized using immunologic precipitation, polyacrylamide gel electrophoresis, amino acid analysis, and radioimmunoassay. Labeling by the lactoperoxidase radioiodination method resulted in selective labeling of the cell surface proteins, as demonstrated by electron microscope autoradiography. A carbohydrate-binding protein (CBP) was found associated with the external surface of the plasma membrane, the appearance of the CBP being a developmentally regulated event. The protein was detected in the soluble protein fraction at 4 hr and on the membrane at 5 hr after the initiation of development. Both RNA and protein synthesis were required for its expression: the synthesis of mRNA coding for the CBP was initiated between 1 and 2 hr of development, and synthesis of the protein was detected at 3-4 hr. CBP was not detectable at 0 and 2 hr of development and it represented only 0.1% of the soluble cellular proteins at 4 hr. Between 4 and 10 hr, a rapid accumulation of CBP was observed until it represented about 1% of the soluble proteins. The concentration of CBP remained constant over the next 6 hr. The time of expression of CBP corresponded to the period when the cells began to acquire intercellular cohesion, and the protein was absent or reduced on the surfaces of three noncohesive mutants. The data suggest that CBP may have a role in cellular cohesion.

310. RECEPTORS FOR HELIX POMATIA A HEMAGGLUTININ ON LEUKEMIC LYMPHOCYTES FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (MEETING ABSTRACT).

Hellstrom U, Mellstedt H, Holm G, Perlmann P
Dept. Immunology, Univ. Stockholm, Stockholm, Sweden
Scand J Immunol; 6(6-7):707 1977

Helix pomatia A hemagglutinin (HP) is a carbohydrate-binding protein with specificity for N-acetyl-D-galactosamine and related sugars. Fluorescein (FITC)-labeled HP binds to subpopulations of human blood lymphocytes treated with neuraminidase. In normal peripheral blood, HP binds to the T lymphocytes, whereas B cells, characterized by surface-bound immunoglobulin (SIg), have no receptors for HP. In chronic lymphocytic leukemia (CLL), HP binds to 90%-100% of the neuraminidase-treated blood lymphocytes. Almost all of the SIg-positive cells in these patients also have receptors for HP. Since CLL is a malignant B-lymphoproliferative disease, these results indicate that HP receptors are only present on immature B cells and on T cells but not on mature B cells. This is also suggested by experiments with peripheral blood from patients with multiple myeloma, containing elevated numbers of SIg-positive cells. In these patients the frequency of HP-positive cells is reduced, and all SIg-positive cells are HP-negative. Alternatively, in CLL HP may react with disease-associated glycoprotein and/or glycolipid present on the leukemic cells. CLL patients in remission with decreased numbers of leukemic cells also have lower numbers of blood lymphocytes carrying both SIg and HP receptors. Testing for HP marker and SIg in combination therefore provides a valuable new method for monitoring patients with CLL. (no Refs)

311. THE BINDING OF MALTOSE TO "VIRGIN" MALTOSE-BINDING PROTEIN IS BIPHASIC.

Hazelbauer GL
Wallenberg Laboratoriet, Uppsala Universitet, Box 562, S-751 22 Uppsala, Sweden
Eur J Biochem; 60(2):445-449 1975

The binding of maltose to maltose-binding protein was investigated in a strain of Escherichia coli, (HfrG6) known to be partially constitutive for a maltose-binding protein. The E coli was grown to late log phase in minimal glycerol salts containing 0.1 mg/ml histidine. The 'virgin' maltose-binding protein was isolated from these cells by osmotic shock, purified on quaternary aminoethyl-Sephadex and Sephadex G100. This material was found to contain less than 5% maltose bound. Exposure of the protein to maltose revealed a biphasic curve having apparent Kd of 1.6 microM and 13 microM. The unusual binding characteristics are thus not due to an isotopic dilution by unlabeled, retained ligand.

312. THE PERIPLASMIC GALACTOSE RECEPTOR PROTEIN OF ESCHERICHIA COLI IN RELATION TO GALACTOSE CHEMOTAXIS.

Kalckar HM
Harvard Medical School, Boston, MA 02114
Biochimie; 58(1-2):81-85 1976

The periplasmic galactose receptor protein of Escherichia coli, the common macromolecule in the initiation of chemotaxis and active transport, was investigated. The galactose receptor protein has a high affinity for free glucose or galactose; with KD's of 2 x 10**-8 and 1 x 10**-7 M, respectively. A second binding site shows a 100-fold lower affinity. The higher concentration of the galactose receptor protein in the periplasmic space tends to retain substrates by recapture of the ligands. The kinetic properties of the galactose receptor protein are generally in harmony with the kinetics of chemotactic responses to spatial or temporal sugar gradients.

E. Catecholamine and Polypeptide Hormone Receptors

313. PROLACTIN RECEPTORS IN HUMAN BREAST TUMORS (MEETING ABSTRACT).

Morgan L, Raggatt PR, de Souza I, Salih H, Hobbs JR
Dept. Chemical Pathology, Westminster Medical Sch., London, SW1P 2AR, England
J Endocrinol; 73(3):17P-18P 1977

The presence of prolactin receptors in human breast carcinomas is reported. Significant specific binding was found in 15/55 breast tumors. The specific binding ranged from 0.9 to 8.8%/mg protein. When these tumors were tested for prolactin dependence in short-term culture, 35 were prolactin-dependent and specific prolactin-binding was found in 13 of them, a 37% correlation. Twenty tumors were independent of prolactin in culture, and of these, only one showed any specific prolactin binding a 95% correlation. The dissociation constant of the high-affinity specific binding was estimated at 10**-8 mole/liter by Scatchard analysis. Human growth hormone, estradiol-17 beta and human chorionic gonadotrophin did not compete for binding with prolactin, but human placental lactogen showed a 20% competition at 1 gammag/ml. Pre-incubation of tumor homogenate with an antiserum raised against rabbit mammary gland prolactin receptors caused abolition of specific prolactin binding. (5 Refs)

314. PROLACTIN IN RAT MAMMARY TUMOUR TISSUE RESPONDS TO HORMONE MANIPULATION (MEETING ABSTRACT).

Cole EN, Nicholson RI
Tenovus Inst. for Cancer Res., Welsh Natl. Sch Medicine, Heath, Cardiff, CF4 4XX, Wales
J Endocrinol; 72(1):35P-36P 1977

The response of prolactin in dimethylbenzanthracene (DMBA)-induced rat mammary tumors to hormone manipulation was investigated. Rapidly raising the plasma prolactin concentration by perphenazine (PZ) treatment (1 mg/rat, iv) resulted in a four-fold increase of tissue prolactin after 1 hr in supernatant fractions derived from homogenate (H) and bicarbonate extract (E). Ovariectomy on the previous day had no effect on prolactin in fraction H but increased it in fraction E from 47 +-5.1 to 72 +-7.4 nanograms prolactin/g protein in the homogenate. Ovariectomy seemed to delay or reduce the prolactin response to PZ in fraction E, while no effect was observed in fraction H after 1 hr. A highly significant correlation between available estradiol receptor concentration and prolactin in fraction E was noted for ovariectomized control rats. Administration of estradiol-17b (5 ug, iv) or tamoxifen (100 ug, iv) produced contrasting effects on tissue prolactin in both fractions; after 24 hr, estradiol resulted in a 50% decrease in the prolactin content, whereas the antiestrogen tended to increase prolactin. In a comparison of control and androgen-treated rats, an association between plasma prolactin and tissue prolactin was observed. This correlation was restricted to fraction E from rats treated im with 1 mg/day of testosterone or androst-5-ene-3b, 17b-diol for 1, 3, and 5 days. Tumors with high prolactin content tended to come from rats with low plasma prolactin concentration. The results observed after PZ administration indicate that the immunoreactive material from rat mammary tumor tissue is prolactin, with further evidence for this being provided by the responses to endocrine manipulation. The correlation of tissue prolactin with estradiol binding is consistent with the known hormone dependency typical of DMBA-induced tumors and suggests that prolactin receptors may be implicated in the hormone content of supernatant fraction E. The paradoxical relationship between plasma and tissue prolactin in androgen-treated rats is readily explained as an example of steroid environment regulating prolactin receptors. (5 refs)

315. STUDIES ON THE ISOLATION AND PURIFICATION OF PROLACTIN RECEPTORS. (PP. 105-115)

Shiu RP, Friesen HG
Cell Membrane Receptors for Viruses, Antigens and Antibodies, Polypeptide Hormones, and Small Molecules, Miles International Symposium Series, Number 9. Beers RF, Bassett EG, ed. New York, Raven Press, 1976.

The isolation and purification of prolactin (PN) receptors from rabbit mammary were studied. By combining differential centrifugation, detergent extraction, and affinity chromatography, the receptor was purified approx 1,500x from rabbit mammary glands. The binding of PN was dependent on time and temperature in the partially purified receptor and the particulate membrane receptors. The most difficult problem in the purification of the receptors was the minute quantity of receptors present in the tissue. The technique used enabled approx 0.5 mg receptor protein per 100 g mammary gland to be obtained. Analysis by isoelectric focusing and gel electrophoresis indicated that the product was not homogeneous. It is concluded that much effort is necessary in order to achieve yields and purities of the PN receptors needed for physicochemical studies. (19 refs)

316. PROLACTIN RECEPTORS AND ANDROGEN-INDUCED REGRESSION OF 7,12-DIMETHYLBENZ(A)ANTHRACENE-INDUCED MAMMARY CARCINOMA.

Costlow ME, Buschow RA, McGuire WL
Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284
Cancer Res; 36(9):3324-3329 1976

Prolactin reverses the inhibitory effects of pharmacological doses of androgen on 7,12-dimethylbenz(a)anthracene-induced mammary tumor growth (Quadri, S K, Kledzik, G S and Meites, J J Natl Cancer Inst, 52: 875-878, 1974). To determine whether this effect is due to an alteration in the ability of the tumor cell to bind prolactin, we have quantitated prolactin receptors in androgen-responsive and nonresponsive tumors. Prolactin receptors were measured with ^{125}I-labeled ovine prolactin in a subcellular fraction which reproducibly contained 60 to 80% of the total receptor present in tumor homogenates. Prolactin binding was reversible, reached a steady state in 9 hr, and was completed by excess unlabeled prolactin. Prolactin bound to its receptor with a Kd of approximately equal to 1 X 10**-10 M. Growing tumors were biopsied, and rats bearing regrown tumors were given injections of 4 mg testosterone propionate twice a week. Prolactin receptors were reduced in most of the tumors, which regressed after testosterone treatment by an average of 63% compared to the pretreatment biopsy specimens. Nonresponsive tumors and vehicle-injected controls showed no significant alterations in receptor content. This reduction of prolactin receptors is probably insufficient to account for androgen-induced mammary tumor regression. (Author Abstract)

317. BRIEF COMMUNICATION: AUTORADIOGRAPHIC LOCALIZATION OF PROLACTIN RECEPTORS IN 7,12-DIMETHYLBENZ(A)ANTHRACENE-INDUCED RAT MAMMARY CARCINOMA.

Costlow ME, McGuire WL
J Natl Cancer Inst; 58(4):1173-1175 1977

Prolactin receptors were localized by autoradiography in 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors from rats by incubation of tumor slices with (^{125}I)ovine prolactin. Specific prolactin binding was confined to tumor cells, and nonspecific binding was present in alveolar spaces and connective tissue. In some tumors, all cells contained receptors; in others, up to one-half the cells remained unlabeled. These results suggested that variation in receptor content in DMBA-induced mammary tumors may be caused by two distinct populations of cells, one which contains receptors and another which possesses very few receptor sites or none at all. (12 Refs) (Author Abstract)

318. BIOLOGY OF PROLACTIN.

Cowie AT, Forsyth IA
Natl. Inst. Res. Dairying, Shinfield, Reading, RG29AT, England
Pharmacol Ther Part B; 1(3):437-457 1975

The functions of prolactin in mammals and some other organisms are reviewed. The numbers of prolactin-secreting cells vary within and among species. Bioassay methods for prolactin include the pigeon crop sac assay, lactogenic responses to prolactin in the rabbit, luteotropic responses in the rat or mouse, and miscellaneous responses such as its ability to release b-glucuronidase activity in rat testis homogenates or to alter behavior in lower vertebrates. Radioimmunoassay methods, including radioreceptor assays and densitometric measurements, are also available. The major biologic responses to prolactin fall into five main categories: effects related to reproduction; effects on osmoregulation and the cardiovascular system; effects related to growth and metabolism; effects on integumentary or ectodermal structures; and effects related to synergism with steroids. It also appears to affect the immune system. Current concepts of the action of protein hormones on target cells envisage that the hormone first binds to specific high-affinity receptor sites on the target cell membrane, after which the hormone-receptor complex activates hormone-dependent processes in the cell. Prolactin is often elevated by pituitary adenomas, in patients with galactorrhea, and in response to a variety of stresses. It is generally tumorigenic, but may inhibit or retard carcinogenesis. Prolactin secretion is controlled by a prolactin-inhibiting factor, a prolactin-releasing factor, thyrotropin, hypothalamic biogenic amines, estrogens, a number of drugs, milking stimulus, its own negative feedback, nonspecific stresses, and circadian rhythms. The levels of prolactin in the plasma of man, cattle, goats, sheep, rats, and mice under varying conditions are reported.

319. AN APPROACH TO LABELING PROTEIN HORMONE RECEPTORS FOR SCANNING ELECTRON MICROSCOPY (SEM) (MEETING ABSTRACT).

Nemanic MK, Borst D, Cammisuli S, Busse WD
Cancer Res. Lab., Dept. Bacteriology-Immunology, Dept. Biochemistry, Univ. California, Berkeley, CA 94720
J Cell Biol; 70(2/Part2):161a 1976

Visualization of the distribution of cell surface hormone receptors should provide new insight into hormonal control of cell function. Bovine insulin and ovine prolactin were modified with hapten molecules for use with the hapten-sandwich method in an attempt to label receptors for these hormones on mouse mammary tumor cells. Insulin containing a chemical bridge between the A1-glycine and B29-lysine was modified with p-diazoniumbenzene arsenate to produce azoars-CBM-insulin; des-PheB1- CBM-insulin was modified with DNP-Phe- nitro-phenyl ester to produce DNP-CBM-insulin. After hapten modification, the CBM group was removed with CNBr in 70% formic acid. Ovine prolactin was modified with azo-ars-p-OH- methyl-benzimidate to yield an average of 3 haptens per prolactin molecule. In a radio-receptor assay using I^{125}-hormone and rat liver membranes, the DNP-insulin and the azo-ars-insulin were 50-60% and 15-20% as active as unmodified insulin, in a similar assay, ars-rolactin was 50-65% as active as unmodified prolactin. Mouse mammary tumor cells, dissociated in saline A plus EDTA (0.025%), possess an average of 10-20x10^3 insulin receptors (KD=5x10**-9) and 3-5x10^3 prolactin receptors (KD=5x10**-10) per cell. Tumor cells maintained in monolayer culture for 3 days in the presence of 10 ug/ml of insulin retained both their initial insulin receptor density and receptor KD. These hapten-modified hormones will be used with anti-hapten antibody and hapten-modified tobacco mosaic virus to label insulin and prolactin receptors on cultured mammary tumor cells.

320. RESPONSES OF MAMMARY CELLS TO HORMONES.

Banerjee MR
International Review of Cytology; Bourne, G. H.; Danielli, J. F.; Jeon, K. W., eds. (New York: Academic Press): vol 47, pp. 1-97; 1976

The responses of mammary cells to hormones are reviewed. Insulin has been observed to increase in vitro DNA synthesis and associated macromolecular events above that in an insulin-free medium. Alveolar cell proliferation and related macromolecular activity have been shown to be regulated by ovarian hormones and by the pituitary in the mammary gland in vivo. Therefore, it has been proposed that the mitogenic responses of mammary cells with respect to insulin action in a culture medium may differ from those in vivo. The results

obtained from both organ culture and in vivo investigations have indicated that prolactin activates RNA synthesis in mammary cells. In conclusion, the availability of an organ culture system in a serum-free medium makes mammary gland systems suitable for studying the regulation of steroid hormone receptor proteins. (501 refs)

321. HORMONE RESPONSIVE HUMAN BREAST CANCER IN LONG-TERM TISSUE CULTURE: EFFECT OF INSULIN.

Osborne CK, Bolan G, Monaco ME, Lippman ME
Medicine Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014
Proc Natl Acad Sci USA; 73(12):4536-4540 1976

The mechanisms of steroid and peptide hormone action in human breast cancer are poorly understood. We have previously characterized a cell line of human breast cancer in long-term tissue culture that possesses various steroid hormone receptors and responses, providing a model for the study of steroid hormone action. The present studies describe a human breast cancer in vitro that responds to physiologic concentrations of insulin with an increased rate of macromolecular synthesis and growth. Thymidine and uridine incorporation in cells in serum-free medium are stimulated by 10**-11 M insulin and are maximal with 10**-8 M. Leucine incorporation is stimulated by 5 x 10**-11 M insulin and is maximal with 10**-9M. Significant stimulation of uridine and leucine incorporation in evident by 3 hr and maximal by 10 hr. A 10-hr lag period exists for insulin stimulation of thymidine incorporation, which is maximal from 14 to 24 hr. The effect of 10**-8 M insulin on macromolecular synthesis is accompanied by a 69% increase above controls in the number of cells after 24 hr. The effect on macromolecular synthesis is observed in glucose-free medium. Insulin's effect on protein synthesis is not blocked by inhibition of RNA synthesis with actinomycin D. Glucocorticoids partially inhibit the action of insulin in these cells. This system provides a model for studying insulin action, and suggests that some human breast cancer may show growth regulation by insulin. (Author Abstract)

322. ECTOPIC BETA-ADRENERGIC RECEPTOR BINDING SITES. POSSIBLE MOLECULAR BASIS OF ABERRANT CATECHOLAMINE RESPONSIVENESS OF AN ADRENOCORTICAL TUMOR ADENYLATE CYCLASE.

Williams LT, Gore TB, Lefkowitz RJ
Dept. Medicine, Duke Univ. Medical Center, Durham, NC 27710
J Clin Invest; 58(2):319-324 1977

The possible molecular basis for the aberrant catecholamine responsiveness of the adenylate cyclase of adrenocortical carcinoma 494 was assessed. The binding of the beta-adrenergic antagonist, ^{3}H-dihydroalpreno'ol, was investigated in membranes prepared from normal adrenal and tumor tissue from male Sprague-Dawley rats. In the presence of 0.7 nanoM antagonist, 48 femtomoles of antagonist were specifically bound per mg of tumor membrane protein. By contrast, membranes from normal adrenal tissue bound only 5 femtomoles antagonist. Experiments were designed to determine whether the binding sites detected in adrenal tumor membranes had the characteristics of beta-adrenergic receptors. Binding of 0.7 nanoM antagonist to 0.3 mg protein/ml of tumor membranes was rapid, reaching a steady-state level in less than 5 at 37 C. Binding was constant for at least 16 min of incubation. In separate experiments, the reversibility of binding was tested by adding a large excess (10**-5M) of propranolol to an equilibrated mixture of antagonist and tumor membranes. The specific binding was rapidly and totally reversible. Analysis of the antagonist binding sites in the tumor membranes demonstrated that they had a high affinity for the antagonist. The number of binding sites (0.094 picomole/mg protein) on the tumor membranes was calculated. The binding displayed a typical beta-adrenergic specificity. The adenylate cyclase of the tumor tissue was stimulated by ACTH, sodium fluoride, and isoproterenol. The results indicate that adrenocortical carcinoma 494 membranes contain beta-adrenergic receptor-binding sites not normally present in adrenal tissue membranes. (21 Refs)

323. CATECHOLAMINE HORMONE RECEPTOR DIFFERENCES IDENTIFIED ON 3T3 AND SIMIAN VIRUS-TRANSFORMED 3T3 CELLS.

Sheppard JR
Dight Inst for Human Genetics, Dept of Genetics and Cell Biology, Univ of Minnesota, Minneapolis, Mn. 55455
Proc Natl Acad Sci USA; 74(3):1091-1094 1977

Identification and characterization of hormone receptors on the cell surface is an effective tool for studying the plasma membrane. Using the direct binding of a radiolabeled antagonist, (-)(^{3}H)alprenolol, to crude membrane preparations, and a physiological response (cellular cyclic AMP levels), I demonstrated a catecholamine (beta-adrenergic) hormone receptor site coupled to a catecholamine responsive adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) on 3T3 and simian virus 40 (SV40)-tranformed 3T3 cells. At a concentration of 1 uM, epinephrine and isoproterenol elevate cellular cyclic AMP levels 8- and 12-fold, respectively, in both cell lines. Norepinephrine was also a potent agonist on 3T3 cells (8-fold stimulation) but SV3T3 cells showed a lesser (2-fold) response to this hormone. The specificity of the physiological response (as well as the direct binding studies using the alprenolol radiolabel) is indicated by the increased effectiveness of (-) compared to (+) stereoisomers, rapid and reversible kinetics (steady state within 2 min), high affinity (Kd approximately 30 nM) and saturability (indicating a finite number of hormone receptors). These hormone receptor studies indicate the 3T3 cells have a beta1 adrenergic receptor while the SV3T3 cells have a receptor with beta2 qualities. In addition, the number of beta-adrenergic hormone receptors appear to be increased in the normal 3T3 cells by approximately 2-fold over the SV3T3 cells (300 versus 120 femtomole/mg of protein). (31 Refs) (Author Abstract)

324. MELANOMA CELLS RESISTANT TO INHIBITION OF GROWTH BY MELANOCYTE STIMULATING HORMONE (MEETING ABSTRACT).

Pawelek J, Sansone M, Koch N, Christie G, Halaban R, Hendee J, Lerner AB, Varga JM
Yale Univ, Sch. Medicine, New Haven, Conn. 06510
Proc Natl Acad Sci USA; 72(3):951-955 1975

Melanoma cells resistant to inhibition of growth by melanocyte stimulating hormone (MSH) were isolated and the relationships of cyclic AMP to the control of both the growth and melanization of these cells were determined. Cells from the Cloudman S91 mouse were cultured in a medium containing MSH (0.2 microM). Melanotic and amelanotic clones that grew in the presence of MSH were isolated and subcloned further to form clones mel-1 and amel-1. The parental strain from which these lines were derived is designated PS1-wt (wild type). MSH or dibutyryl cyclic AMP (Bt2AMP, 250 microM) caused growth inhibition and morphological changes in wild-type cells but not in amel-1 or mel-1 cells. Wild-type cells had high basal tyrosinase activity which was stimulated further by MSH or Bt2AMP; amel-1 cells had very low basal tyrosinase activity which was not stimulated by MSH or Bt2AMP. The mel-1 cells also had very low basal tyrosinase activity but retained MSH or Bt2AMP

inducible activity. There was somewhat more MSH receptor activity in amel-1 and mel-1 cells than in wild-type cells. Bt2AMP caused an increase in MSH receptor activity in mel-1 and wild-type cells but usually decreased the activity in amel-1 cells. All three lines contained melanosomes. Endogenous cAMP levels, cAMP-dependent protein kinase activity, cAMP binding proteins, and cAMP transport were essentially the same for all three lines. Wild-type and mel-1 cells showed increased intracellular cAMP levels in response to MSH and or theophylline; amel-1 cells did not. It is concluded that tyrosinase activity present in the absence of added MSH is related through a common biochemical pathway to MSH control of growth. However, MSH-inducible tyrosinase activity does not appear to be related to MSH control of growth. The morphological changes that occur following the addition of MSH or cAMP to melanoma cells are related to growth of the cells and not to melanization.

325. PRESENCE IN NORMAL HUMAN TESTES OF A CHORIONIC-GONADOTROPIN-LIKE SUBSTANCE DISTINCT FROM HUMAN LUTEINIZING HORMONE.

Braunstein GD, Rasor J, Wade ME
Cedars-Sinai Medical Center, 4833 Fountain Ave., Los Angeles, Calif. 90029
N Engl J Med; 293(26):1339-1343 1975

The possibility that normal human testes contain small amounts of human chorionic gonadotropin was investigated. Extracts of human testes obtained at autopsy demonstrated parallel inhibition curves to the human chorionic gonadotropin standard in a radioimmunoassay specific for the hormone. The immunoreactive material was adsorbed onto concanavalin A, a reaction characteristic of glycoproteins, and was eluted within the chorionic gonadotropin range on Sephadex G-100 column chromatography. Solubilized receptor proteins for the hormone could not be identified in the extracts. The demonstration that the normal human testes contain a glycoprotein similar or identical to human chorionic gonadotropin suggests that the fetal genome responsible for production of the hormone during pregnancy is not completely suppressed in the adult. Excessive production of this glycoprotein may account for the high levels of human chorionic gonadotropin reported in the serum of patients with germ-cell tumors of the testes.

326. ERYTHROPOIETIN AND THE DIFFERENTIATION OF RED BLOOD CELLS.

Goldwasser E
Department of Biochemistry, The University of Chicago, and The Franklin McLean Memorial Research Institute, Chicago, Illinois 60637
Fed Proc; 34(13):2285-2292 1975

Erythropoietin is the primary factor regulating red blood cell formation in mammals and some other animals. It has been purified from plasma derived from anemic sheep and from the urine of anemic patients and is a glycoprotein. The sheep hormone has a molecular weight of 46,000 and appears to consist of a single chain. The carbohydrate and amino acid compositions where known, are summarized, as are the known structural requirements for biological activity. Its mode of action as the inducer of red cells differentiation has been studied in marrow cell and fetal liver cell cultures. Erythropoietin interacts first with a protein receptor on its target cell causing the appearance of a cytoplasmic protein thought to be a mediator that, in turn, causes an increased rate of transcription in the target cell nuclei. There are several different species of RNA synthesized before the cells initiate hemoglobin synthesis. The effect on transcription is only indirectly dependent on DNA synthesis. Some properties of the primitive erythropoientin-responsive cell are discussed and a cellular model for blood cell differentiation is presented. (Author Abstract)

327. THE EFFECT OF 12-0-TETRADECANOYL PHORBOL ACETATE (TPA) ON THE METABOLISM OF 3',5'-CYCLIC AMP (CAMP) IN MOUSE SKIN (MEETING ABSTRACT).

Mufson RA, Simsiman RC, Boutwell RK
McArdle Lab, Univ of Wis, Madison, WI 53706
Proc Am Assoc Cancer Res; 17:132 1976

The effects of the tumor promoter TPA on the regulation of cAMP levels in mouse skin are being studied. Epidermal-dermal preparations were flaked from the frozen (-70 degrees C) backs of mice. The cAMP was extracted into 5% TCA, purified on Dowex 50H**+ columns, and measured with a protein binding assay. No significant change from basal level was detected either 5-15 min or 1-24 hrs after topical application of 17 nmols TPA. The same result was obtained with epidermal preparations. It was found that 9-24 hrs after TPA treatment the ability of isoproterenol (0.3 micromol), epinephrine (0.3 micromol) or phenylephrine (3.0 micromol) to stimulate the beta-receptor and increase the cAMP levels of skin cells was significantly diminished or completely abolished. Further, compared to acetone controls the isoproterenol stimulated cAMP accumulation in TPA treated skin had an increased sensitivity to beta-adrenergic blockade by propranalol. In vitro assay of adenyl cyclase activity revealed that after homogenization the enzyme from either TPA or acetone treated epidermis could be stimulated by isoproterenol to the same degree (33%). These results suggest that TPA does not act directly through altering the basal cAMP level, but by altering the properties of membrane receptors. (Author Abstract)

328. DIFFERENCES BETWEEN PURIFIED ECTOPIC AND NORMAL ALPHA SUBUNITS OF HUMAN GLYCOPROTEIN HORMONES.

Weintraub BD, Kr uth G, Rosen SW, Rabson AS
Veterans Admin. Hosp., Prescott, Ariz. 86301
J Clin Invest; 56(4):1043-1052 1975

"Ectopic" proteins, not distinguished immunologically from the common alpha subunit of the human glycoprotein hormones, were purified approx 10,000-fold from a gastric carcinoid tumor (A.L-alpha) and from tissue culture medium of bronchogenic carcinoma cell lines (ChaGo-alpha). The purified A.L-alpha was homogeneous by sodium dodecyl sulfate (SDS) gel electrophoresis while the purified ChaGo-alpha showed multiple components, some of which represented aggregated species. In SDS gel electrophoresis, the apparent molecular wt of A.L-alpha (15,000) and dithioerytaritol-reduced ChaGo-alpha (13,000) were significantly lower than those of the alpha subunits of human chorionic gonadotropin (hCG-alpha), luteinizing hormone, follicle-stimulating hormone, or thyroid-stimulating hormone (22,000-23,000). Binding experiments with ^{35}S-SDS suggested that these apparent differences in molecular wt resulted, at least in part, from diminished binding of the SDS by the normal compared to the ectopic alpha subunits. In gel chromatography, the apparent molecular wt of A.L-alpha (27,000) and ChaGo-alpha (30,000) were slightly higher than those of normal alpha subunits (23,000-24,000). Both A.L-alpha and ChaGo-alpha were not distinguished from hCG-alpha in ion-exchange chromatography. The composition of A.L-alpha was similar to that of hCG-alpha in 13 amino acids but showed decreased phenylalanine and increased valine; glucosamine was identified in both A.L-alpha and hCG-alpha. Under conditions in which hCG-alpha combined with the hCG beta subunit (hCG-beta) to produce 95% of the expected gonadotropin-binding activity in a rat testis radioreceptor-

assay. A L-alpha incubation with hCG-beta resulted in only 2% of the expected activity, and ChaGo-alpha incubation with hCG-beta produced no detectable activity.

329. MEMBRANE RECEPTORS AND HORMONE ACTION.

Cuatrecasas P, Hollenberg MD

Advances in Protein Chemistry. Anfinsen CB, Edsall JT, Richards FM, ed. New York, Academic Press, 1976 1:90022E-56 + + +

Several aspects of the direct study of membrane-localized hormone receptors are discussed. Significant progress has been made over the past 10 yr in the direct analysis of the interaction of hormones with membrane-localized receptors. Information concerning the mobility and cellular location of cell-surface receptors for immunoglobulins has been obtained. It has also been possible to solubilize membrane receptor structures and apply the techniques of protein chemistry for their isolation and physicochemical characterization. Several aspects are reviewed: the relation between biological activity and binding; the physicochemical detection and assay of receptors; the solubilization of membrane receptors; examples of membrane-localized receptors; affinity techniques; polyvalent hormone derivatives; receptor studies; and the theoretical mechanisms of hormone-receptor binding and action.

F. Other Cell Surface Binding Sites

330. STRUCTURE AND FUNCTION OF IMMUNOGLOBULIN GENES AND IMMUNOGLOBULIN PRECURSORS.

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Ann Immunol (Paris) 127C(3-4):421-435 1976

The capacity of a distinct V region probe to hybridize and quantify V genes of the same and different subgroups was examined to gain information on the origin of antibody diversity (somatic mutation) or germ cell hypothesis). The extent of cross-hybridization of a distinct L chain complementary DNA (cDNA) with mRNAs coding for L chains of the same and different subgroups was studied. The results indicated that V regions of similar amino acid sequence are coded by similar nucleotide sequence and that the nucleic acid probe to one V region may anneal and quantify V genes of members of the same subgroup. Molecular hybridizations of the cDNA probe with nuclear DNA showed that the number of kappa type C genes is small, the number of V genes is also reasonably small, and there is no amplification of these genes in myeloma cells that produce large amounts of the immunoglobulin (Ig). These results support the somatic mutation model for the generation of antibody diversity. New information on the structure and functional expression of Ig genes was obtained from the study of L chain precursors. Using the tiny amounts produced of L chain mRNA translation in vitro, the precursor (pre) peptide segments were identified and found to be extensions of the amino (N) terminus of secreted L chains. Amino acid sequence analyses of the precursors provided evidence that the gene coding for the V region is larger than hitherto known: duplication of a short DNA segment is carried in the structural gene coding for the pre(V)C (pre-cursor) translation of the L chain messenger RNA may be continuous on the polysome ensemble coding for the entire pre- sequence at the exact point where post-translational cleavage occurs. Using the cDNA probe to investigate the expression of Ig genes in myeloma cells, it was found that the genes for the V region of the L chain and the H chain are expressed in the same cell in a manner similar to the synthesis of the membrane and secreted forms of membrane-bound proteins. It is proposed that most precursor molecules are directed to the endoplasmic reticulum where the extra piece is cleaved to yield mature Ig destined for secretion; a few precursor molecules escape cleavage and are anchored by means of the hydrophobic extra piece in the cell-surface membrane to serve as the antigen-recognizing receptor.

331. LYMPHOCYTE-MEDIATED CYTOLYSIS OF ALLOGENEIC TUMOR CELLS IN VITRO LYMPHOCYTE-TUMOR CELL BINDING AND MEMBRANE BINDING SITES.

Todd RF

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Diss Abstr Int B 36(10):4939-B 1976

The binding stage of the interaction between cytotoxic (T) lymphocytes (CL) and target cells was studied, particularly the membrane binding sites on both CL and target cells. The lysis of Cr51-labeled EL4 tumor target cells by BALB/c anti-EL4 serum was inhibited by hyperimmune BALB/c anti-EL4 serum due to the prevention of specific PE -EL4 binding. Pretreatment of PEL with trypsin reversibly inhibited their capacity to lyse EL4 and to bind to EL4 monolayers. Papain and pronase were also inhibitory while neuraminidase was the opposite. There was no IgM on the surface of PEL. The CL receptor could not be found in EPL subcellular fractions. Determinations of the H-2 content of target cells after enzyme treatment showed approx 80% of the surface H-2 was removed by papain or pronase, 40% by trypsin and none by neuraminidase. PEL did not bind to nonviable cells or to particulate or soluble target membrane fractions with strong H-2 activity. PEL could not bind to EL4 monolayers in the presence of 2,4-dinitrophenol and sodium azide. It is proposed that the CL receptor is a protein which either represents a minute fraction of the membrane surface, making its detection difficult, or it is highly labile. (Auth)

332. IN VITRO TOLERANCE INDUCTION OF NEONATAL MURINE B CELLS.

Metcalf ES, Klinman NR

Dept. Pathology, Sch. Medicine, Univ. Pennsylvania, Philadelphia, PA 19174

J Exp Med 143(6):1323-1340 1976

The susceptibility of neonatal and adult BALB/c mouse B lymphocytes to tolerance induction was analyzed by a modification of the in vitro splenic focus technique. Neonatal or adult spleen cells were adoptively transferred into irradiated syngeneic adult recipients that had been carrier-primed to hemocyanin (Hy), thus maximizing stimulation to the hapten 2,4-dinitrophenyl coupled to Hy (DNP-Hy). Cultures were initially treated with dinitrophenyl (DNP) or several heterologous (non-Hy) carriers and subsequently stimulated with DNP-Hy. The responsiveness of adult B cells was not diminished by pretreatment with any DNP conjugate; however, most of the neonatal B cell response was abolished by in vitro culture with all of the DNP/protein conjugates. During the first week of life, the ability to tolerize neonatal splenic B cells progressively decreased. Thus tolerance in this system is restricted to B cells early in development, accomplished by both tolerogens and immunogens, achieved at low (10^{-8}-8 M determinant) antigen concentrations, and is highly specific, discriminating between DNP- and TNP-specific B cells. It is concluded that B lymphocytes mature through a stage in which they are extremely susceptible to tolerogenesis. The specific interaction of B cell antigen receptors with multivalent antigens is tolerogenic to neonatal (immature) B cells unless antigens concomitantly recognized by primed T cells and macrophages can counteract the susceptibility of immature and mature B lymphocytes. (II. Tolerance induction suggests intrinsic differences between neonatal and adult B cells and may

provide a physiologically relevant model for the study of tolerance to self-antigens. (54 refs)

333. EXCITEMENT OVER B2-MICROGLOBULIN

Ralf MC
No affiliation given
Nature (Lond); 254(5498):287-288, 1975

Observations suggesting an evolutionary link between B2-microglobulin, immunoglobulin (Ig), and the major histocompatibility (H) antigens are presented. The complete amino acid sequence of human B2-microglobulin shows a striking degree of homology with the constant domains of human IgG. The findings suggest that B2-microglobulin represents a free Ig domain with effector function similar to the terminal domain of IgG. Two independent lines of evidence indicate that B2-microglobulin is an integral part of the subunit structure of all the membrane proteins which carry the serologically defined major histocompatibility antigens of the surface of all normal nucleated cells. The light chain of HL-A appears identical with B2-microglobulin, which noncovalently binds to the heavy chains in a structure similar to Ig. Studies employing the binding of multivalent ligands to specific determinants on cell surfaces reveal that the association of solubilized HL-A with B2-microglobulin is not an artifact of the solubilization or separation procedures. The structure of B2-microglobulin suggests it is found in the membrane only through interacting with other membrane proteins. It is found associated with thymus leukemia antigens and teratocarcinoma alloantigens, both of which are determined by genes closely linked to the H-2 complex. While not true for all alloantigens coded for by genes in the H-2 complex, it is possible that the B2-microglobulin serve to stabilize the functional conformation of the membrane polypeptides. Despite the apparent lack of genetic linkage, homology in structure between B2-microglobulin and some Ig domain suggests the possibility of evolution from a common ancestral gene. The physical association of B2-microglobulin with some of the T cell receptor proteins suggests an evolutionary link between B-cell-mediated humoral immunity and T-cell-determined cell-mediated immunity. The possible immunological importance is extended by recent findings that anti-B2-microglobulin antibodies inhibit various T cell responses, although the true function of B2-microglobulin remains unknown. (19 refs)

334. SPECIFIC BINDING OF NERVE GROWTH FACTOR (NGF) BY MURINE C-1300 NEUROBLASTOMA CELLS.

Rosenthal R, Bertolini L, Pedrini M, Vigneti E
Ist. Cell Biol., Natl. Res. Council, Rome, Italy
J Exp Med; 140(2):445-451, 1974

The nerve growth factor (NGF), a protein capable of inducing differentiation of sympathetic nerve cells, binds specifically to the membrane surface of murine C-1300 neuroblastoma cells. The specific binding capacity of NGF by the cells was quantitatively measured by a radioimmunoassay technique. At 4°C a certain number of the molecules of NGF could be bound at equilibrium by each cell with an average relative association constant of about 10^9 liters/mol. Experiments with trypsinized cells showed that either the number of molecules of NGF bound or the affinity of the binding mechanism for NGF, and self-aided depending on their presence or absence during the first hours of the interaction between NGF and the cells. Binding of ^{125}I-NGF was abolished by trypsin treatment of the cells, new receptors were, however, soon regenerated, and the membrane surface again became able to bind NGF. Experiments carried out at 37°C and in the presence of complement showed that the cells bound NGF and then lysed. Cells treated with trypsin, which had lost their ability to bind NGF, were also resistant to the lytic effect of NGF. Cells which had been treated with NGF and then trypsinized were found to be

inhibits the action of diphtheria toxin in HeLa cells, demonstrating that the B fragment of the toxin probably exerts the initial interaction with the cell membrane. Since the S-sulfonated B chain, even at 1.3 ug/ml, cannot inhibit the action of toxin on HeLa cell cultures, the sulfonyl groups may prevent the B chain from binding to the receptor site. Reconstitution of nicked toxin from isolated A and B chains has been unsuccessful, using a number of methods. However, a technique was developed by which radioactive (125I) toxin was specifically labeled in either the A or the B chain. Modifications of diphtheria toxin with specific reagents revealed that alterations of the tryptophan, methionine, and arginine residues did not lead to a significant loss in toxicity. Treatment with o-phthalaldehyde or by photooxidation with rose bengal results in a complete loss of toxicity. Modification of tyrosine by iodination yields active toxin; modification by tetranitromethane causes a loss of activity. (32 refs)

337. CELL SPREADING FACTOR. OCCURRENCE AND SPECIFICITY OF ACTION.

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Exp Cell Res. 102(1):51-62 1976

A factor required for spreading of substratum-attached baby hamster kidney (BHK) cells, Chinese hamster ovary cells, HeLa cells and L cells was isolated and purified from fetal calf serum. A similar factor was also found in calf, porcine, human, rabbit, and chicken sera. The factor was active when adsorbed to the substratum. Prior adsorption of other proteins prevented cell spreading. Antibody against the fetal calf spreading factor inhibited the spreading activity of the unfractionated fetal calf serum, the calf serum, and porcine serum. Antibody against BHK cell surfaces induced cell spreading when the antibody was adsorbed to the substratum. Cell spreading was not observed when the antibody against BHK cell surface was present in the incubation medium and the substratum. It is proposed that this spreading factor is specific and that it adsorbs to the substratum surface, thereby being activated. It then forms the target for certain cell surface receptors. Interactions between adsorbed-activated factor and cell surface receptors leads to cell spreading. (38 Refs)

338. SEPARATION, IDENTIFICATION AND MECHANISM OF ACTION OF THE GRANULOCYTIC CHALONE.

Paukovits WR, Paukovits JB
Inst. Cancer Res., Univ. Vienna-Borschkegasse 8a, 1090 Vienna, Austria
Boll Ist Sieroter Milan. 54(3):177-186 1975

Highly purified granulocytic chalone from bone marrow conditioned medium was prepared and characterized. A sequence of gel-chromatographic steps on Sephadex G-25, G-15 and Biogel P-6 was applied. The end product had a sigmoid dose-response-curve with a plateau at 40% to 50% inhibition of thymidine incorporation. The inhibition was only observed with bone marrow cells as target cells; lymphoid cells were not affected. When bone marrow cells were treated for 1 min with 0.08% trypsin and subsequently used for assaying chalone activity in the presence of cycloheximide, no inhibition was found. These results may indicate that chalone specific receptor sites or protein feature are present on the surface of the target cells, which are destroyed by the trypsin treatment. Granulocytic chalone was soluble in aqueous solvents and in polar organic solvent mixtures. Its activity was destroyed by trypsin but only after prolonged treatment. The elution behavior of chalone is discussed in detail. Chromatography on Sephadex G-25 and Ultrafiltration point to a molecular wt of several thousands, whereas chromatography on G-25 or G-15 (from Biogel P-6) indicated a rather low molecular wt. (27 refs)

339. DIFFERENTIATED FUNCTIONS OF SCHWANN CELL TUMOR CLONES (MEETING ABSTRACT).

Wechsler W
Max-Planck Inst. fuer Hirnforschung, 5000 Koeln-Merheim, Ostmerheimerstrasse 200, West Germany
J Cell Biol. 70(2 Part2):216a 1976

In order to study normal and pathological functions of mammalian Schwann cells at the cellular and molecular level various tumor clones have been established as permanent cell lines by isolation with the alternate-animal-culture-technique from experimental nerve tumors. The primary tumors were induced systemically in inbred rats (BD IX and CDF strains) and mice (C3H, C57Bl, DBA and A strains) by ethylnitrosourea either transplacentally during fetal gestation or neonatally. The neoplastic Schwann cell clones isolated represent cell types with various patterns of differentiation or anaplasia. The morphology of the clones was studied both in vitro and in vivo after animal transplantation by light, transmission and scanning electron microscopy. Rat and mouse Schwann cell tumor clones are highly mobile with respect to locomotion, movement of processes and pulsation. Rat Schwann cell tumor clones are capable of synthesizing proteins characteristic for the nervous system, e.g. S-100, 14-3-2, GFA or D2; in addition they are involved in collagen biosynthesis. They lack surface receptors for isoproterenol and norepinephine neurohormones, but possess histocompatibility and cell specific surface antigens. Membrane potentials are dependent on cellular contacts. Furthermore a relative high activity of CNPase was found regularly, while a basic protein detected in RN-2 and RN-6 clones could so far not be identified as a basic myelin protein by chemical, immunological and animal experiments.

340. SURFACE MODULATION IN CELL RECOGNITION AND GROWTH: SOME NEW HYPOTHESES ON PHENOTYPIC ALTERATION AND TRANSMEMBRANOUS CONTROL OF CELL SURFACE RECEPTORS.

Edelman GM
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Science 192(4236):218-226 1976

The author hypothesizes that the processes of cell growth, movement, and recognition are coordinated by interaction of surface receptors and cytoplasmic fibrillar structures. These processes are thought to be regulated by alterations in the structure, mobility, and distribution of specific cell surface receptors. Cell surface glycoproteins appear to play a major role in these surface modulations. Lymphocytes and embryonic chick neural cells are discussed as models for this hypothesis. The H-2 histocompatibility antigen is thought to be involved in immune recognition, rejection, and lysis. A T lymphocyte recognition system requiring a viral antigen-histocompatibility antigen complex is presented as a model for lymphocyte lysis of tumor cells. Experiments with chick embryonic retinal cells indicate that cell-cell interactions may be controlled by surface proteins that are activated by proteolysis of high molecular weight glycoproteins. A surface modulating assembly consisting of glycoprotein receptors, microfilaments, and microtubules is presented as a model for anchorage modulation. This modulation is thought to be involved in stabilization of receptor sites and transmembrane control of such cellular processes as movement, division, and cell-cell interactions. The question of how cells coordinate signals for these processes is discussed with respect to the author's hypotheses.

341. STIMULATION OF LIVER MICROSOMAL SIALYLTRANSFERASE ACTIVITY BY LYSOLECITHIN.

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FEBS Lett; 62(2):165-168 1976

The stimulation of liver microsomal sialyltransferase activity by lysolecithin was investigated. Egg yolk lysolecithin stimulated the transfer of sialic acid from CMP-sialic acid to DSF by rat liver microsomes by a factor of up to 6.5 with maximum stimulation in the concentration range of 2 to 8 mM. This stimulatory activity was not caused by the esterified fatty acid moiety, the choline moiety, nor the L-alpha-lysophosphatidyl moiety. The stimulatory effect of lysolecithin was observed at all concentrations of the CMP-sialic acid donor and of the glycoprotein receptor examined. It is proposed that the lysolecithin could stimulate sialyltransferase activity by solubilizing the enzyme, thus facilitating the interaction of the enzyme with the macromolecular acceptor.

342. GLUTEN-SENSITIVE ENTEROPATHY. (PP. 208-214)

Strober W, Falchuk M, Gebhard RI

Metabolism Branch, Natl. Cancer Inst., Bethesda, Md. 20014

Immunodeficiency in Man and Animals. Bergsma D, Good RA, Finstad J, Paul NW, ed. Sunderland, MA, Sinauer Associates, Inc., 1975

Gluten-sensitive enteropathy (GSE) is a disease characterized by villous atrophy related to the ingestion of wheat protein, gluten. The rates of immunoglobulin A (IgA) and IgM synthesis in the jejunums of patients with GSE were studied, as was the in vitro effect of gluten protein on the epithelial cells of GSE patients in remission and cells from the same patients following gluten challenge. HL-A frequency was studied in patients with GSE and dermatitis herpetiformis. Gluten ingestion in patients with GSE was promptly followed by a local immune reaction involving the production of anti-gluten antibodies. Using an in vitro model of gluten enteropathy involving the organ culture of biopsy tissue, it was demonstrated that gluten is not directly toxic to the gastrointestinal mucosa; instead, it brings about tissue damage via the activation of an endogenous mechanism, presumably the immune system. About 90% of GSE patients carried the HL-A8 histocompatibility type, as compared with 60-65% of patients with dermatitis herpetiformis. HL-8A may be a marker for the presence of an abnormal immune response gene or it may determine the presence of abnormal gluten-protein receptor sites on epithelial cells. Either of these abnormalities could result in a propensity to respond immunologically to gluten, with destructive consequences.

IV. DNA AND RNA BINDING PROTEINS

A. DNA and RNA Binding Proteins in Viral Infection and Transformation

343. CHARACTERIZATION OF AN ADENOVIRUS EARLY PROTEIN REQUIRED FOR VIRAL DNA REPLICATION: A SINGLE STRAND SPECIFIC DNA BINDING PROTEIN.

Levine AJ, van der Vliet PC, Rosenwirth B, Anderson C, Rabek J, Levinson A, Anderson S

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Mol Cell Biochem; 11(2):79-95 1976

1. The human adenoviruses types 2, 5 and 12 code for the production of a single strand specific DNA binding protein. The molecular weights of these proteins were 72,000 for types 2 and 5 and 60,000 for type 12. In all three cases proteolytic breakdown fragments of these binding proteins (48,000 MW) were also observed. 2. Analysis of the methionine containing tryptic peptides of these proteins indicate that the types 2 and 5 proteins are similar and clearly distinguishable from the type 12 protein. The peptide maps of these three viral proteins are clearly different from a similar protein found in mock infected cells. 3. Temperature sensitive mutants of type 5 (H5ts125) and type 12 (H12tsA275) adenoviruses fail to produce these proteins at the nonpermissive temperature. H5ts125 infected cells grown at the permissive temperature produce a 72,000 MW protein that is thermolabile, for continued binding to DNA, when compared to type 5 wild type adenovirus 72,000 MW protein. An analysis of the phenotype of this adenovirus mutant indicates that it codes for a viral function at early times after infection that is required for viral DNA replication. 4. The in vitro translation of adenovirus specific m-RNA results in the synthesis of a small amount of a 72,000 MW protein that binds to single stranded DNA just like the authentic adenovirus DNA binding proteins produced in infected cells. 5. Adenovirus anti-Tumor antigen (T) antiserum from hamsters carrying independently derived adenovirus tumors, have been tested for the presence of antibody to purified DNA binding proteins. One antiserum is positive for these antibodies while the other is negative. These results indicate that some, but not all, adenovirus tumors contain large enough levels of the DNA binding proteins to elicit an antibody response. 6. The type 5 adenovirus temperature sensitive mutant, H5ts125, that codes for a thermolabile DNA binding protein, was complemented or suppressed at the nonpermissive temperature, for the replication of adenovirus DNA, by SV40. SV40 tsA temperature sensitive mutants, defective in SV40 DNA replication, do not suppress or complement H5ts125 at the nonpermissive temperature. (Author Abstract)

344. EARLY EVENTS IN ADENOVIRUS-INFECTED CELLS. (PP. 551-557)

Russell WC

Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, NY); vol. 39, 1975

Serological, polypeptide synthesis, and temperature-sensitive mutant studies on adenovirus-infected cells are reviewed. Serological studies suggest that adenovirus-derived tumors have components that are related to both nonstructural and structural (possibly fiber) antigens. Recent evidence suggests that core proteins are synthesized early in infection, and since the synthesis of cellular histones is inhibited later in infection, it is suggested that the basic core polypeptides may play a role in the replication of virus DNA somewhat analogous to that of histones. If infected cell-specific polypeptides and the core polypeptides are associated with the replication of viral DNA, they may be DNA-binding proteins. The early findings that broad tumor sera react type-specifically with fiber antigen can be explained by the possibility that some of the reactions against structural components can be related to antibody against the core components (probably made early in infection), or the possibility that the fiber antigen preparations were not rigorously purified. These studies indicate that only a few possible viral gene products can be detected early in a lytic infection or in an abortive infection. Some or all of these products appear to be concerned with initiating and maintaining viral DNA synthesis; failure to do so may lead to integration of the viral genome into the cellular chromosome. The integrated viral genome would probably express a viral gene function that would kill the host; in a few cases, the functions that are integrated or expressed may be compatible with the

coexisting cellular functions and thus could lead to transformation and oncogenesis.

345. LOCATION AND IDENTIFICATION OF THE GENES FOR ADENOVIRUS TYPE 2 EARLY POLYPEPTIDES.

Lewis JB, Atkins JF, Baum PR, Solem R, Anderson CW
Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724
Cell (Camb); 7(1):141-151 1976

Virus-specific RNA was prepared from transformed rodent cell line 8617, and the F17 and 14b cell lines, early after adenovirus type 2 infection, and fractionated by hybridization to specific fragments of viral DNA. The viral mRNA was used to program cell-free protein synthesis, and the products were analyzed by electrophoresis. The genes for the early polypeptides of apparent molecular weight 44,000, 15,000, 72,000, 15,500, 19,000, and 11,000 daltons were located, respectively, between positions 0-4.1, 4.1-16.7, 58.5-70.7, 75.9-83.4, 89.7-98.6, and 89.7-98.6 of the conventional adenovirus DNA map. The polypeptide of molecular weight 72,000 daltons was the previously described single-strand DNA-binding protein. RNAs from three different adeno-transformed cell lines each programmed the synthesis in vitro of the 15K polypeptide, predominantly, as well as variable amounts of the polypeptide of molecular weight 44,000 daltons. The genes for these two polypeptides were located in the portion of DNA required for transformation of rodent cells by adenovirus.

346. CHARACTERIZATION OF AN ADENOVIRUS EARLY PROTEIN REQUIRED FOR VIRAL DNA REPLICATION: A SINGLE STRAND SPECIFIC DNA BINDING PROTEIN.

Levine AJ, van der Vliet PC, Rosenwirth B, Anderson C, Rabek J, Levinson A, Anderson S
Princeton Univ. Dept. Biochemical Sciences, Princeton, NJ 08540
Mol Cell Biochem; 11(2):79-95 1976

An attempt was made to characterize the single-strand-specific binding proteins of human adenovirus types 2, 5 and 12. Results indicated that the molecular weights of these proteins were 72,000 for types 2 and 5 and 60,000 for type 12. In all three cases proteolytic breakdown fragments of these binding proteins (mol wt 48,000) were also observed. Analysis of the methionine containing tryptic peptides of these proteins indicated that the types 2 and 5 proteins were similar and clearly distinguishable from the type 12 protein. The peptide maps of these three viral proteins were clearly different from a similar protein found in mock infected cells. Temperature sensitive mutants of type 5 (H5ts125) and type 12 (H12tsA275) adenoviruses failed to produce these proteins at the non-permissive temperature. H5ts125 infected cells grown at the permissive temperature produced a protein (mol wt 72,000) that was thermolabile for continued binding to DNA, when compared to type 5 wild type adenovirus protein (mol wt 72,000). An analysis of the phenotype of this adenovirus mutant indicated that it coded for a viral function early in the infection that was required for viral DNA replication.

347. LOCATION AND IDENTIFICATION OF THE GENES FOR ADENOVIRUS TYPE 2 POLYPEPTIDES (MEETING ABSTRACT).

Atkins JF, Lewis JB, Anderson CW, Gesteland RF
Dept. Molecular Biology, Univ. Edinburgh, Edinburgh, Scotland, UK
Biochem Soc Trans; 4(5):859 1976

The identification and location of the genes for adenovirus type 2 polypeptides are reported. Cytoplasmic RNA isolated from KB cells after infection by adenovirus type 2 was fractionated by hybridization to specific fragments of adenovirus DNA produced by cleavage with restriction endonucleases. Viral messenger RNA was used as a template for protein synthesis in cell-free mammalian extracts. The genes for the early polypeptides of apparent molecular wt 52,000 (44,000), 15,000, 72,000, 15,500, 19,000, and 11,000 were located between positions 0-4.1, 4.1-16.7, 58.5-70.7, 75.9-83.4, 89.7-98.6 and 89.7-98.6, respectively, of the conventional adenovirus map. The polypeptide of molecular wt 72,000 was shown to be the single-strand DNA-binding protein described by others. A partial map of the late genes of adenovirus type 2 was similarly constructed, and the products coded by the viral RNA in three transformed cell lines were analyzed. (3 refs)

348. THE RELATIONSHIP BETWEEN GROUP C ADENOVIRUS TUMOR ANTIGEN AND THE ADENOVIRUS SINGLE-STRAND DNA BINDING PROTEIN.

Levinson A, Levine AJ, Anderson S, Osborn M, Rosenwirth B, Weber K
Princeton Univ. Dept. Biochemical Sciences, Princeton, NJ 08540
Cell (Camb); 7(4):575-584 1976

The single-strand specific DNA-binding protein (mol wt 72,000 daltons) produced by group C adenoviruses soon after productive infection is studied to determine whether it was expressed in adenovirus tumors and transformed cells. Two independently derived preparations of antisera from hamsters bearing group C adenovirus tumors were tested for antibody against the single-stranded DNA-binding proteins. One antiserum contained antibodies that reacted with these DNA-binding proteins, while the second antiserum did not contain detectable levels of antibody. Five adenovirus type 2 transformed rat cell lines were tested for the presence of the single-strand specific DNA-binding proteins. Two of the five transformed cells (T2 C4 and F-4) expressed detectable levels of this protein. These results indicate that the group C adenovirus single-strand specific DNA-binding proteins are expressed in some, but not all, adenovirus tumors and transformed cell lines. Those transformed cell lines (type 2) containing a portion of the adenovirus genome designated by the Eco R-I-B restriction enzyme fragment expressed the single-strand specific DNA-binding proteins, while those cell lines missing it did not contain this protein. Other experiments have located the structural gene of the single-strand specific DNA-binding protein in the Eco R-I-B DNA fragment indicating that when this gene is present in a transformed cell it is expressed.

349. ADENOVIRUS TYPE 2 CODED SINGLE-STRANDED DNA BINDING PROTEIN: IN VIVO PHOSPHORYLATION AND MODIFICATION.

Jeng YH, Wold WS, Sugawara K, Gilead Z, Green M
Inst for Molecular Virology, Saint Louis Univ Sch of Medicine, St Louis, MO 63110
J Virol; 22(2):402-411 1977

The adenovirus type 2-coded single-stranded DNA binding protein (DBP) was shown to be a phosphoprotein and to exist in at least two forms that differ in mobility by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After a 30-min pulse with [^{35}S]methionine or $^{32}PO_4$, ^{35}S- or ^{32}P-labeled DBP had a nominal molecular weight of 74,000, whereas after a 30-min label followed by a 20-h chase, ^{35}S- and ^{32}P-labeled DBP had a nominal molecular weight of 77,000. Both large and small forms of ^{35}S- and ^{32}P-labeled DBP bound to single-stranded DNA-cellulose columns and were eluted by 0.4 to 0.6 M NaCl; both forms also were immunoprecipitated by antiserum against adenovirus type 1-simian virus 40-induced tumor cells (this antiserum contains antibodies against DBP) and by monospecific antiserum against 95 to 99% purified DBP. With highly purified ^{32}P-DBP labeled 7 to 10 h

postinfection, it was shown that the ^{32}P radioactivity was firmly associated with protein material (ie, not contaminating nucleic acids or phospholipids) and had properties expected of a phosphoester of an amino acid; paper electrophoresis of acid hydrolysates of this preparation identified phosphoserine but not phosphothreonine. Phosphoserine but not phosphothreonine was also identified in acid hydrolysates of another preparation of ^{32}P-DBP labeled for 30 min, chased for 20 h, and then immunoprecipitated by adenovirus type 1-simian virus 40 antiserum. (34 Refs). (Author Abstract)

350. TRYPTIC FINGERPRINT ANALYSIS OF ADENOVIRUS TYPES 2, 5 AND 12 DNA-BINDING PROTEINS.

Rosenwirth B, Anderson C, Levine AJ
Inst. Virology, Cologne, W. Germany
Virology; 69(2):617-625 1976

Tryptic fingerprints of the peptides derived from ^{35}S methionine-labeled DNA-binding proteins produced by human adenovirus (Ad) at early times after infection of human or monkey cells were obtained. These included a 72,000 molecular weight (MW) protein produced by Ad2, a 72,000 MW protein produced by Ad5, and a 58,000 protein produced by Ad12. A collection of 2 or 3 lower MW (41,000-47,000) single-strand-specific DNA-binding proteins, also produced after infection, were also analyzed. The 72,000-MW (Ad2 and 5) and 58,000-MW (Ad12) proteins synthesized in adenovirus-infected cells were found to be distinct from a 72,000-MW cellular DNA-binding protein found in uninfected or mock-infected cells. The ^{35}S methionine-labeled peptides derived from the 41,000-47,000-MW proteins were found to be similar, if not identical, to the ^{35}S methionine-labeled tryptic peptides derived from the larger DNA-binding proteins. The ^{35}S methionine-labeled peptides derived from the Ad2 and 5, 72,000-MW proteins were quite similar but distinct from the Ad12, 58,000-MW protein. The peptide fingerprints of the Ad2, 72,000-MW protein synthesized in either human or monkey cells were very similar or identical.

351. DNA BINDING PROTEINS IN THE CYTOPLASM AND IN A NUCLEAR MEMBRANE COMPLEX ISOLATED FROM UNINFECTED AND ADENOVIRUS 2 INFECTED CELLS.

Shanmugan G, Bhaduri S, Arens M, Green M
St. Louis Univ. Sch. Medicine, Mo. 63110
Biochemistry (Washington); 14(2):332-337 1975

The DNA-binding proteins in a nuclear membrane fraction that can synthesize DNA in vitro and in the cytoplasm of adenovirus infected and uninfected cells were isolated and characterized. Suspension cultures of human KB cells infected with human adenovirus 2 (100 plaque-forming U/cell) were treated with 25 microg/ml of arabinosylcytosine and then labeled with (3H)leucine from 6-24 hr postinfection. Uninfected cells were treated similarly and labeled with (14C)leucine. The 3H-labeled and 14C-labeled proteins isolated from the cytoplasm were mixed, as were corresponding proteins isolated from the membrane complex. DNA-binding proteins were selected by adsorption in 0.05 M NaCl to single-stranded DNA-cellulose columns and fractionated by elution with 0.15, 0.4, 0.6, and 2 M NaCl. A major fraction of the labeled proteins from the nuclear membrane complex (50-60%) and a small amount from the cytoplasm (10-20%) bound to DNA cellulose in 0.05 M NaCl. Larger quantities of DNA binding proteins were present on the nuclear membrane complex and in the cytoplasm of infected cells than in the corresponding fractions from uninfected cells. The electrophoretic profile of the 0.15 and 0.4 M NaCl eluates from the nuclear membrane complex revealed a heterogenous mixture of polypeptides in infected and uninfected cells. The 0.6 M eluate contained two major components of molecular wt 75,000 and 45,000 that were present only in infected cell preparations. These two proteins were also present in the same eluate from the cytoplasm of infected cells. Two major cell specific components of molecular wt 40,000 and 15,000-17,000 were present in the 2 M eluate of the membrane complex. One major cell-specific protein of molecular wt 33,000 was present in the 0.15 and 0.4 M eluate of the cytoplasm of infected and uninfected cells. Analysis of cells labeled in the absence of arabinosylcytosine indicated that synthesis of the DNA binding proteins of molecular wt 75,000 and 40,000 begins early after infection prior to the onset of viral DNA replication. It is suggested that the two DNA binding proteins in adenovirus 2-infected cells reflect the expression of the parental viral genome.

352. CELL TRANSFORMATION: A STUDY OF REGULATION WITH TYPES 5 AND 12 ADENOVIRUS TEMPERATURE-SENSITIVE MUTANTS. (PP. 419-426)

Ginsberg HS, Ensinger MJ, Kauffman RS, Mayer AJ, Lundholm U
Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology : Cold Spring Harbor Lab. (Cold Spring Harbor, NY): vol. 39, 1975

The types 5 and 12 temperature sensitive (ts) mutants of adenovirus (Ad) that cannot replicate their DNA under nonpermissive conditions (39.5 C) are described. In addition, one that can make the major viral gene products but under nonpermissive conditions cannot transport its hexon protein into the nucleus for virion assembly is characterized. The following insights were achieved: (a) viral DNA replication requires at least two specific viral proteins; (b) hexon protein transport requires a nonhexon viral protein; (c) virion assembly cannot ensue if one of the viral gene products is defective; and (d) transformation is the consequence of abortive infections with many adenoviruses, but its incidence is increased after infection with one class of adenovirus ts mutants. Two models are presented to explain the last observation. In one model, the DNA-binding protein normally maintains the viral DNA in a specific conformation or attached to some cellular structure so that viral genome integration is prohibited and viral DNA replication is initiated and continues. In the absence of this protein integration is more commonly permitted. In the second model, viral genome integration into a host chromosome is a normal event in the initial stages of infection, even during a productive infection. The latter hypothesis suggests that in a normal productive infection the entire viral genome is excised and replicated; defective excision occurs in abortively infected cells, leaving genome fragments to initiate and maintain transformation.

353. ADENOVIRUS-INFECTED, CELL-SPECIFIC, DNA-BINDING PROTEINS. (PP. 559-566)

Levine AJ, van der Vliet PC, Rosenwirth B, Rabek J, Frenkel G, Ensinger M
Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor Lab. (Cold Spring Harbor, NY): vol. 39, 1975

Cell-specific DNA-binding proteins were isolated from adenovirus-infected cells and characterized. African green monkey kidney (AGMK) cells were mock-infected or infected with type 5 adenovirus (Ad5) and labeled with ^{14}C-leucine or ^{3}H-leucine. Two infected, cell-specific, single-stranded DNA-binding proteins were produced with molecular weights of 72,000 and 48,000 daltons. Similar proteins were found in Ad2- and Ad12-infected AGMK cells. The purified DNA-binding proteins bound preferentially and cooperatively to any single-stranded DNA tested, but no binding to double-stranded DNA or 23S Escherichia coli RNA was detected. Approximately 1 to 10 x 10^5 molecules were synthesized after infection of the AGMK cells, suggesting a stoichiometric

rather than a catalytic function for these proteins. Extracts of Ad5 ts 125-infected AGMK cells prepared at the nonpermissive temperature did not contain detectable adenovirus DNA-binding proteins, but these proteins were present in extracts prepared at the permissive temperature. The amount of DNA-binding proteins consistently decreased when Ad5 ts 125-infected AGMK cells were shifted from the permissive to the nonpermissive temperature. The proteins produced at permissive temperatures appeared to be more thermolabile for binding to single-stranded DNA than wild-type proteins. It is possible that the 48,000-dalton protein is a proteolytic breakdown product of the 72,000-dalton protein. SV40 infection of AGMK cells was able to overcome the Ad5 ts 125 block to adenovirus DNA synthesis. SV40 ts B-8 and SV40 ts B-11 mutants, but not SV40 ts A-30, were also able to complement Ad5 ts 125 in AGMK cells. The data suggest that the adenovirus-induced DNA-binding proteins are required for adenovirus DNA replication. Common functional aspects of adenovirus and SV40 cellular transformation are also suggested.

354. THE ISOLATION AND CHARACTERIZATION OF DNA BINDING PROTEINS SPECIFIC FOR ADENOVIRUS INFECTED CELLS. (PP. 197-216)

van der Vliet P, Levine AJ

Tumor Virus-Host Cell Interaction; Edited by A. Kolber. (New York: Plenum Press): 1975

The structure and replication of adenovirus DNA isolated from virions of adenovirus types 2 and 5 (Ad2 and Ad5, respectively) are reviewed, and two adenovirus-infected cell-specific DNA binding proteins are characterized. Monolayer cultures of African green monkey kidney cells were infected with Ad5, and ^{3}H-leucine (10 uCi/ml) was added to the infected cells 2 hr later. A fraction of the protein extracts was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Two prominent polypeptides, detected only in infected cells, were observed the mol wt of which were estimated to be 72,000 and 48,000 daltons. Each was found to be present in amounts between 1-10 x 10^{5} molecules/cell. The kinetics of synthesis of these two DNA binding proteins was investigated by labeling adenovirus-infected African green monkey kidney cells with ^{14}C-leucine for 4-hr periods at various times after infection, fractionating the protein extracts on DNA cellulose, and analyzing the proteins by electrophoresis. Both proteins were first detected at about 8 hr after infection and were synthesized at a maximum rate at 18 hr. Neither protein could be detected in preparations of purified virions. Both were detected in extracts prepared from cells infected with a DNA-negative temperature-sensitive mutant, Ad5 ts36, grown at the permissive or nonpermissive temperatures. Neither protein was detected in cell extracts prepared from cells infected with a DNA-negative temperature sensitive mutant Ad5 ts225 grown at the nonpermissive temperature. Thirty percent of the normal yield of these proteins was present in Ad5 ts225 infected cell extracts prepared at the permissive temperature. Both proteins bind to single-stranded DNA but not to double-stranded DNA or RNA. All of these properties closely resemble those of the previously described T-4 gene 32 product, but their function is not clear.

355. TUMOUR ANTIGEN SPECIFICITY OF A DNA-BINDING PROTEIN FROM CELLS INFECTED WITH ADENOVIRUS 2.

Gilead Z, Arens MQ, Bhaduri S, Shanmugam G, Green M

St. Louis Univ. Sch. Med., Mo.

Nature (Lond), 254(5500):533-536 1975

A new DNA-binding polypeptide of molecular weight 75,000 (75k protein) in cells infected with adenovirus 2 (Ad2) was studied by three immunological methods. Most of the 75k protein purified from infected cell cytoplasm by single-stranded DNA-cellulose column chromatography was present in the fraction eluting in 0.6 M NaCl. Unlabeled 0.6 M eluates were assayed by complement fixation using hamster sera against several tumor antigens. Positive fixation to a titre of 1:32 occurred with anti-Ad 2 and anti-Ad 1 tumor sera. Anti-Ad 12 tumor serum, anti-SV40 tumor serum, and goat antiserum to purified Ad 2 virions were all negative. Controls using mock-infected cells were negative. An indirect radioimmune precipitation assay showed a maximum 63%-66% of the input radioactivity of the labeled 0.6 M eluate precipitated with anti-Ad 1 and anti-Ad 2 immunoglobulins, whereas only 7%-10% was precipitated by the immunoglobulins from the anti-SV40 or goat anti-Ad 2 virion sera. In radioimmune-inhibition experiments, the precipitation of ^{3}H -labeled protein from the 0.6 M eluate with a saturating level of anti-Ad 2 tumor immunoglobulin could be inhibited by 80% by preincubating the immunoglobulin with increasing amounts of unlabeled 0.6 M eluate or unlabeled infected cytoplasm. The results indicate that the 75k DNA-binding protein is a non-virion, group C-specific tumor antigen found only in infected cells.

356. ISOLATION AND CHARACTERIZATION OF ADENOVIRUS TYPE 12 DNA BINDING PROTEINS

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Inst. Virology, Univ. Cologne, Cologne, Germany

Virology, 67(1):14-23 1975

The isolation of a set of single strand-specific DNA binding proteins from adenovirus type 12-infected African green monkey kidney (AGMK) cells is described, and the binding proteins are characterized. The proteins were labeled with ^{3}Hleucine , ^{14}Cleucine , or ^{35}Smethionine , after which the cell extract was centrifuged and the supernatant containing the DNA binding proteins was applied to a DNA-cellulose column containing single-stranded calf thymus DNA. The binding proteins thus eluted were dialyzed against buffer and added to single or double-stranded type 12 adenovirus DNA . The DNA binding proteins were also subjected to sodium dodecyl sulfate -gel electrophoresis and autoradiography. Infection of the AGMK cells with adenovirus type 12 resulted in the production of two single-stranded specific DNA binding proteins of molecular weight 60,000 and 48,000, respectively. Both proteins were synthesized in the absence of viral DNA replication and neither protein appeared to correspond to any polypeptide detected in mature adenovirus virions. Temperature-sensitive mutants from three different early, DNA-negative complementation groups (tsA, tsB, and tsC) were also tested for the production of these proteins at the permissive and nonpermissive temperature. Mutants of the tsB and tsC classes produced both DNA binding proteins at both 32 and 40 C, while the tsA mutants produced both proteins at 32 C but neither protein at 40 C. The results suggest that the adenovirus type 12 gene induces or codes for the DNA binding proteins, and that adenoviruses in general produce a single strand-specific DNA binding protein that is required for viral DNA replication.

357. REGIONAL LOCALIZATION OF HUMAN GENES IN VIRUS-INDUCED UNCOILER REGIONS.

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Dept. Cancer Studies, Univ. Birmingham, Birmingham, Conn.

Cytogenet Cell Genet, 14(3-6):202-204 1975

Previous studies on adenovirus 12-infected mouse-man hybrid cells containing only the long arm and the centromere region of human chromosome 17 enabled localization of the thymidine kinase (TK) gene to the virus-induced uncoiler region. In the present study, the localization of galactokinase (GaK) of an adenovirus 12-infected hybrid cell line derived by

Sendai virus-mediated fusion of normal human fibroblasts and mouse L cells deficient in TK was investigated. Subclones previously shown to have lost TK activity and the portion of the chromosome 17 distal to 17q21 were negative for human GaK. Those subclones retaining a larger portion of the long arms, but which are deleted distal to the break in 17q22, retained both human TK and GaK activity. Thus, the genes for both these enzymes must be located in the uncoiler regions between the two break points and are therefore assigned to human chromosome 17, band q21-22. Studies are in progress to determine whether a virus-coded DNA -binding protein may be the mediator of the uncoiling effect.

358. THE STRUCTURE AND REPLICATION OF THE ADENOVIRUS GENOME. (PP. 125-141)

Pearson GD

The Biology of Tumor Viruses Proceedings of the Thirty-fourth Annual Biology Colloquium. Beaudreau GS, Synder S, ed. Corvallis, Oregon State University Press, 1976.

The DNA structure and replication of the closely related types 2 and 5 adenoviruses are reviewed. The linear adenovirus chromosome has a MW of 23 x 10⁶. The sequence of the chromosome, determined by denaturation mapping, is not circularly permuted. Site-specific endonucleases cleave adenovirus DNA into unique fragments, permitting the topography of the chromosome to be defined completely. Adenoviruses replicate in the nuclei of infected cells. Viral DNA most likely exists within the nucleus as a nucleoprotein complex that contains two virus-specified DNA binding proteins as well as endonuclease and DNA polymerase activities. Adenoviruses replicate discontinuously, the average time required for replication being 26-27 min. Complete joining of daughter strands requires at least an additional 25-30 min. A replication map of adenovirus was constructed using the EcoRI restriction endonuclease. These data together with those from pulse-labeling experiments indicate that two replicative origins are positioned 25% from either end of the chromosome. The light alkaline strand is used as a template for leftward replication starting in fragment C and ending in fragment A. The heavy alkaline parental strand is displaced. There are four origins for rightward synthesis on the displaced strand: one at the left end, one 25% from the left end, one near the middle of the chromosome, and one in EcoRI-F. These distances roughly correspond to the sizes of unjoined daughter strands present in newly finished molecules. The adenovirus molecule is not terminally repetitious in the usual sense, but has an inverted terminal repetition. A model illustrating the structure and role of the inverted terminal repetition in adenovirus DNA indicates that the ends of the linear duplex molecule fold back as hairpins. As a consequence, the nucleotide sequence at the 3' terminus of one strand is not necessarily complementary to the sequence at the 5' terminus of the other strand. Another model for the replication of linear molecules that also involves hairpin ends has been proposed independently. Experiments are in progress to test for the presence of hairpin regions in the adenovirus chromosome. (48 refs)

359. GENETICS OF ADENOVIRUSES.

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Adv. Cancer Res. 23:91-130 1976

The mutagenesis and selection of adenovirus mutants, their use in genetic studies, their phenotypes, and their usefulness in experiments on the regulation of adenovirus replication and cellular transformation are reviewed. Although the adenovirus genome can potentially code for 50 or more proteins, unique mutations have been detected in only 10-12 genes. Of the number of conditionally lethal, temperature-sensitive (ts) mutants isolated, there is considerable clustering of two phenotypes, hexon and assembly mutants. The mutants isolated follow predictive intergenic reactions. The grouping of hexon mutants in a single cistron confirms chemical and physical evidence that the hexon is formed from the assembly of three identical polypeptide chains. Synthesis, assembly, and transport of hexon capsomers require more than a single gene product. The detection of three nonoverlapping complementation groups representing three genes essential for production of a functional fiber implies that the fiber consists of three rather than two unique species of polypeptides. Types 5 and 12 ts mutants have revealed a minimum of three viral proteins essential for initiation of DNA replication, but only one gene product has been identified so far, a DNA-binding protein that prefers single-stranded DNA. Experiments with two of these mutants, H5ts125 and H5ts149, have confirmed that late transcripts can be made only after replication of viral DNA has begun. The three mutants, H5ts125, H12ts307, and H12ts401, transform significantly more cells than wild-type viruses or other ts mutants. H5ts125 has been most thoroughly characterized and, as previously noted, is defective in a virus-specific, single-stranded DNA-binding protein. The protein of the viral genome that codes for the ts 125 gene product, which is approximately 0.65 physical map unit from the left-hand end of the genome, is rarely integrated in adenovirus-transformed cells. Three independently isolated mutants present this same phenomenon of increased transforming capacity, whereas three other DNA-minus mutants, belonging to another complement group (H5ts36 and 37 and H5ts149), show either decreased or normal transformation frequency. It appears that the adenovirus DNA-binding protein plays a regulatory role in transformation as well as in DNA replication. (140 refs)

360. SYNTHESIS OF THE ADENOVIRUS-CODED DNA BINDING PROTEIN IN INFECTED CELLS.

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J Virol; 18(2):454-460 1976

Synthesis of the 75K (mol wt 70,000-75,000) DNA binding protein, an early virus-coded protein in adenovirus 2-infected KB cells, and its regulation were studied by radioimmune precipitation inhibition assay. The protein was first detected 4 hr postinfection and accumulated at an exponential rate. An arrest of further synthesis (accumulation) was observed at 10 to 11 hr postinfection, coinciding with the synthesis of late virion proteins. In contrast, when the infected cells were treated with arabinosyl cytosine (25 ug/ml) to block viral DNA replication, the synthesis of this protein did not cease but continued for up to 36 hr postinfection. The synthesis of 75K protein in cells, after release from a cycloheximide block (2 to 9 hr postinfection) was analyzed. Although increased amounts of early adenovirus-specific mRNA generally accumulate in infected cells during such a block, treatment did not produce increased levels of this protein, and an abrupt arrest of its formation again occurred upon the synthesis of late virion proteins. Partition of the 75K protein between the nuclear and cytoplasmic fractions during the infection was studied. The protein appeared first in the cytoplasm and then in the nucleus after a slight lag. Accumulation of the protein continued both in the cytoplasm and nucleus with higher levels being found in the cytoplasm.

361. THE ISOLATION AND IDENTIFICATION OF THE ADENOVIRUS GROUP C TUMOR ANTIGENS.

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Virology; 76(1):1-11 1977

Two independently derived antisera (14b and Ad1-simian virus 40, SV40) from hamsters bearing distinct adenovirus (Ad) group C-induced tumors were used to immunoprecipitate and identify the tumor antigens produced in Ad-infected cells. Monkey or human cells infected with Ad5 were labeled with ^{35}S-methionine early or late after viral infection. The specific immunoprecipitates were analyzed electrophoretically to determine the molecular wt (MW) of the Ad-infected cell antigens that reacted with the antibody from the antisera. One antiserum preparation (14b) immunoprecipitated predominantly a single protein of 58,000 MW. No homologous protein was detected in uninfected cells, and normal hamster serum failed to immunoprecipitate this antigen from infected cell extracts. The other antiserum (Ad1-SV40) specifically immunoprecipitated this 58,000 MW protein, and it also reacted with viral-specific antigens of 72,000 and 44,000 MW. All three of these viral-specific proteins were synthesized early after infection, prior to viral DNA replication. Late after viral infection, the Ad1-SV40 serum immunoprecipitated or coprecipitated a 120,000 MW protein. The 72,000 MW protein is immunologically related to the Ad single-strand-specific DNA binding protein. Some or all of the 44,000 MW protein resulted from the proteolytic breakdown of the 72,000 MW protein. The Ad5-transformed cell line 14b contained the left-hand 40% of the Ad genome. The early Ad genes carried in the 14b DNA segment are common to all Ad-transformed cell lines. It is therefore likely that the 58,000 MW protein found in Ad-infected cells is an Ad-specific tumor antigen common to all tumors and transformed cells. (25 Refs)

362. MAPPING OF ADENOVIRUS 2 GENES BY TRANSLATION OF RNA SELECTED BY HYBRIDIZATION.

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INSERM; 47:293-298 1975

A biochemical map of adenovirus type 2 DNA was constructed by hybridizing the messenger RNA's (mRNA's) from infected cells to various fragments of the DNA obtained with restriction enzymes and translating the hybridized RNA's. Most of the proteins synthesized in adenoinfected cells at late times after infection are encoded by adeno DNA. Analysis of the polypeptides formed with mRNA's produced at early times after infection indicated that seven specific polypeptides (72K, 52K, 45K, 19K, 15.5K, 15K, and 11K) are synthesized in vitro. Results obtained with the restriction endonuclease, Eco RI, showed that the mRNA for the 72K DNA-binding protein is located on the map in a region of the DNA that is not necessary for transformation. The results suggest, however, that the critical parts of T antigen with respect to transformation should be located in the left-hand end of the map, a region required for establishment and maintenance of the transformed state. (20 Refs)

363. ADENOVIRUS DNA-BINDING PROTEIN IN CELLS INFECTED WITH WILD-TYPE 5 ADENOVIRUS AND TWO DNA-MINUS, TEMPERATURE-SENSITIVE MUTANTS, H5TS125 AND H5TS149.

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J Virol; 23(1):142-151 1977

Studies have been done to characterize further H5ts125, an adenovirus type 5 conditionally lethal, temperature-sensitive (ts) mutant defective in initiation of DNA synthesis and to investigate whether the single-strand- specific DNA-binding (72,000 mol wt) protein is coded by the mutated viral gene. When H5ts125-infected cells were labeled with (S35) methionine at 32C and then incubated without isotope at 39.5C, the mutant's nonpermissive temperature, the 72,000 mol wt polypeptide was progressively degraded. Immunofluorescence examination of cells infected with wild-type virus, H5ts125 and H5ts149 (a second, unique DNA-minus mutant) showed that immunologically reactive DNA-binding protein was barely detectable in H5ts125-infected cells at 39.5C, whereas this protein was present in wild-type- and H5ts149-infected cells, that the protein made at 32C in H5ts125-infected cells lost its ability to bind specific DNA-binding protein antibody when the infected cells l greater than 1 were shifted to 39.5C, and that if H5ts125-infected cells were shifted from the restrictive temperature to 32C, even in the presence of cycloheximide to stop protein synthesis, immunologically reactive DNA-binding protein reappeared. (Author abstract) (20 Refs)

364. IN VIVO AND IN VITRO PHOSPHORYLATION OF THE ADENOVIRUS TYPE 5 SINGLE-STRAND-SPECIFIC DNA-BINDING PROTEIN.

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Virology; 79(1):144-159 1977

Evidence is presented that the 72,000-molecular wt (MW), single-strand-specific DNA-binding protein of adenovirus type 5 can be labeled with ^{32}PO4 added as inorganic phosphate to the medium of infected monkey or human kidney cells. The DNA-binding protein in the infected cell can be isolated as a phosphoprotein at early and late times after infection. Most or all of the ^{32}P-phosphate can be removed from the DNA-binding protein by alkaline phosphatase or hydrolysis at alkaline pH. The dephosphorylated protein retains the ability to bind specifically to single-stranded DNA. Cells infected with the H5ts125 temperature-sensitive mutants of adenovirus type 5, which contains a mutation in the structural gene of the 72,000-MW protein, failed to phosphorylate this protein at the nonpermissive temperature, even though they synthesized approx normal (wild-type) levels of the protein during short labeling times (30 min). An in vitro nuclear protein kinase assay is described that can detect the phosphorylation of three adenovirus-induced phosphoproteins (100,000, 72,000, 36,000 MW) that are also observed when adenovirus-infected cells are labeled with inorganic ^{32}P-phosphate in vivo. The protein kinase activity detected in this in vitro assay is not dependent on or stimulated by the addition of exogenous cyclic AMP or cyclic guanosine monophosphate. The 72,000-MW protein phosphorylated in vitro by this phosphokinase activity has been shown to be the adenovirus single-strand-specific DNA-binding protein by a specific immunoprecipitation test. (24 Refs)

365. IMMUNOFLUORESCENCE STUDY OF THE ADENOVIRUS TYPE 2 SINGLE-STRANDED DNA BINDING PROTEIN IN INFECTED AND TRANSFORMED CELLS.

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J Virol; 22(2):527-539 1977

High-titer monospecific antiserum against highly purified adenovirus 2 (Ad2) single-stranded DNA binding protein (DBP) was used to study, by indirect immunofluorescence (IF), the synthesis of DBP in Ad2-infected human cells and adenovirus-transformed rat, hamster, and human cell lines. In infected cells the synthesis of DBP was first detected in the cytoplasm at 2 to 4 hr postinfection and reached a maximum intensity at 6 hr postinfection. At this time DBP began to accumulate in the nucleus, where it reached maximum intensity at about 14 hr postinfection. The cytoplasmic IF was diffuse, whereas nuclear IF appeared as dots that coalesced into large globules as infection progressed. In cells treated with 1-beta-D-arabinofuranosylcytosine to inhibit viral DNA synthesis, strong nuclear IF was observed in the form of dots, but the large fluorescent globules were not observed. The Ad2 (oncogenic group C) anti-DBP serum reacted very strongly by IF with Ad5 (group C)-infected, to a lesser extent with Ad7 and Ad11 (group B)-infected, and weakly with Ad12 and Ad18 (group A)-infected KB cells (treated with 1-beta-D-arabinofuranosylcytosine). These results may indicate that Ad2 DBP is closely related immunologically to DBPs induced early after infection by adenovirus serotypes in oncogenic group C, moderately related to DBPs of serotypes in oncogenic group B, and perhaps distantly related to DBPs of serotypes in oncogenic group A. The following adenovirus-transformed cell lines were examined for DBP synthesis by IF with the Ad2 anti-DBP serum: six rat cell lines (T2C4, F17, 8662, 8638, 8617, and F161) transformed by Ad2 virus, three hamster cell lines transformed by Ad2 virus (Ad2HT1) and Ad2-simian virus 40 hybrid virus (ND1HK1 and ND4HK4), and one rat (5RK) and one human (293-31) cell line transformed by transfection with Ad5 DNA. T2C4 and 8662 appeared weakly positive, whereas Ad2HT1 and ND4HK1 were strongly positive. The other transformed cell lines did not produce DBP detectable by IF. Thus, some but not all transformed cell lines produce DBP, which indicates that DBP is not required for maintenance of cell transformation and that transformed cells can express "nontransforming" viral genes as protein. (33 Refs) (Author Abstract)

366. THERMOLABILE T (TUMOR) ANTIGEN FROM CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE MUTANT OF SIMIAN VIRUS 40 (MEETING ABSTRACT).

Tenen DG, Baywell P, Livingston DM
Sidney Farber Cancer Center, Boston, Mass. 02115
Proc Natl Acad Sci USA; 72(11):4351-4355 1975

Partially purified tumor (T) antigen from a strain of Chinese hamster lung cells transformed by wild-type Simian virus 40 (SV40) and either of two temperature-sensitive SV40 mutants was studied as a DNA -binding protein. The DNA binding activity present in the T-antigen-containing fractions was inhibited by purified hamster anti-T IgG but not by equivalent amounts of nonimmune hamster IgG. T from either wild-type- or tsC219-transformed cells was relatively stable during heating at 44 C compared to T antigen prepared from tsA239-transformed cells, the latter antigen lost half of its activity in one hour at 44 C and 30% in two hours at 30 C. Antigen from wild-type cells lost less than 10% of its activity during these treatments. These results strongly suggest that T antigen is a product of the SV40 A gene.

367. AN ANALYSIS BY ELECTRON MICROSCOPY OF EARLY SIMIAN VIRUS 40 RNA FROM A TSA MUTANT.

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Cell (Camb); 11(3):523-531 1977

A new electron microscopic technique, developed by J Ferguson and R W Davis (manuscript in preparation), has been used to investigate the pattern of early transcription in cells infected with simian virus 40 (SV40). DNA binding protein from Escherichia coli and antibody to this protein increase the apparent thickness of single-stranded DNA. Thus the positions of termini and the sizes of individual molecules of RNA can be determined after hybridization to a defined single strand DNA. Since the concentration of early RNA is very low in cells infected by wild-type SV40, the RNA was prepared from cells infected with the temperature-sensitive mutant tsA58, where early RNA is greatly over- produced after a shift from permissive to restrictive temperature late in infection. Nuclear or cytoplasmic RNA was hybridized to full-length linear early strands of SV40 DNA. Using the known end points generated by the restriction endonuclease (Eco R1) and an internal marker to define orientation of the strand, the positions of the 5' and 3' termini of individual RNA molecules were determined. Most molecules of cytoplasmic early RNA have a 5' terminus at 0.67 SV40 fractional length, and the majority of the 3' termini extend well into the late region, at least as far as the Eco R1 restriction site at map position 0. The mean 5' terminus of nuclear early RNA molecules was at 0.68 SV40 fractional length. Many of these molecules are small (0.01-0.20 fractional length), but some extend to the Eco R1 restriction site or possibly beyond, as found for the cytoplasmic early RNA. The effects on the composition and size of early RNA pools of the tsA mutation or of exposure to high temperature are unknown. (Author abstract) (23 Refs)

368. DIFFERENCES IN DNA-BINDING PROTEINS ISOLATED FROM NORMAL AND TRANSFORMED HUMAN CELLS.

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Exp Cell Res; 99(1):115-125 1976

The DNA-binding proteins (DBP) of normal human fibroblasts (line W138) and transformed cell lines were compared. The DBP were separated into components by electrophoresis. Three significant differences were observed between W138 cells and simian virus 40-transformed cells. The DBP component P3 was greater in W138 cells and DBP components P5a and P6b were greater in VA13B-2RA cells. Differences in P5a and P6b were not seen in replicating vs stationary phase cultures of W138 cells. Comparison of confluent cultures of W138 and VA13B-2RA cells showed that normal cells were replicating very little but that almost half the transformed cells were replicating. Components P9 and P10 were all present in greater amounts in the SV40 transformed cells, whereas P3 and P5c were greater in the normal cells. P5a and P6b were also present in greater amounts in HeLa cells than in W138 and in chemically transformed human liver cells vs normal liver cells. Thus, these DBP differences do not appear to be specific for transformation by SV40. Increased P5a amounts were detected in 7/9 transformed cell lines examined: VA13B, HeLa, OG, D98, AG, BT-20, 734B and SUSM-1. The two lines which were lacking this P5a change (T151 and T98) were sensitive to density-dependent inhibition of replication. Increases in P5a may therefore play a role in the release from density-dependent inhibition of replication that is observed in most transformed cells. (37 Refs)

369. STIMULATION OF ALPHA, BETA AND HERPES SIMPLEX VIRUS-INDUCED DNA POLYMERASES BY DNA BINDING PROTEINS. (MEETING ABSTRACT).

Blue W, Weissbach A

Roche Inst of Molecular Biology, Nutley, NJ 07110

Fed Proc; 36(3):734 1977

DNA binding proteins in uninfected and herpes simplex virus (HSV) type 1 infected HeLa cells were examined by DNA cellulose chromatography and a sensitive DNA binding protein assay. The DNA binding proteins eluted from single-stranded DNA cellulose by stepwise salt gradients were examined for their ability to stimulate or inhibit the HeLa cell alpha, beta and gamma DNA polymerases or HSV-induced DNA polymerase. Proteins which stimulate either the alpha, or beta, or HSV-induced DNA polymerases were found and each was further purified over phosphocellulose. The partially purified DNA binding proteins exhibit specificity for a given DNA polymerase and neither enhance nor inhibit the other enzymes. Enhancement by the alpha and beta-stimulating proteins occurred regardless of the template used (ie, activated or synthetic DNA's) while stimulation of HSV polymerase by its specific stimulating protein was detected only when utilizing dG12-18-poly(dC) as a template. (Author Abstract)

370. DNA-BINDING PROTEINS OF CELLS INFECTED BY HERPES SIMPLEX VIRUS TYPE 1 AND TYPE 2.

Powell KL, Purifoy DJ

Department of Virology and Epidemiology, Baylor College of Medicine, Texas Medical Center, Houston, TX 77030

Intervirology; 7(4-5):225-239 1976

Proteins showing affinity for DNA in HSV-1- and HSV-2-infected cells were compared by DNA-cellulose chromatography and PAGE. The proteins observed depended on the type of virus used to infect the cell; however, several examples of analogous polypeptides were present in cells infected by both virus types. Proteins showing highest affinity for DNA-cellulose were similar in molecular size in cells infected by both virus types. (16 Refs) (Author Abstract)

371. HERPES SIMPLEX VIRUS PROTEINS: DNA-BINDING PROTEINS IN INFECTED CELLS AND IN THE VIRUS STRUCTURE.

Bayliss GJ, Marsden HS, Hay J

M.R.C. Virology Unit, Univ. Glasgow, Glasgow G11 5JR, Scotland

Virology; 68(1):124-134 1975

The DNA-binding proteins were isolated from BHK21 C13 cells infected with herpes simplex virus type 1 strain 17 syn+ (by native and denatured DNA affinity chromatography). Sixteen proteins were identified as specific to the infected cell on SDS polyacrylamide gradient gel electrophoresis; their molecular wt ranged from 1.45×10^5 to 10.5×10^5. After infection with herpes simplex virus, many of the mock infected cell specific DNA binding proteins were no longer produced; this "switch off" of host cell protein synthesis was rapid. The virus-induced DNA-binding proteins fell into two quantitative classes; there was evidence for temporal control of their synthesis. One of the virus-induced DNA-binding proteins had a molecular wt identical to a polypeptide found in full but not empty particles, another bound preferentially to denatured DNA.

372. DNA-BINDING PROTEINS INDUCED BY HERPES SIMPLEX VIRUS TYPE 2 IN HEP-2 CELLS.

Purifoy DJ, Powell KL

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030

J Virol; 19(2):717-731 1976

Affinity chromatography on single-stranded and double-stranded DNA-cellulose indicates that 12 proteins previously identified from herpes simplex virus type 2-infected cells, ranging in molecular weight from 28×10^5 to 186×10^5, bind to DNA-cellulose. The DNA-binding proteins found in infected cells differed in relative binding strengths for denatured DNA-cellulose. The virus specificity of these DNA-binding proteins was further studied by comparison with DNA-binding proteins isolated from mock-infected cells, and by immunoprecipitation of infected-cell DNA-binding proteins with antisera specific for viral antigens. The promise this technique holds for the purification and study of polypeptides involved in virus DNA replication, recombination, or repair is discussed. (Author Abstract)

373. DEOXYRIBONUCLEIC ACID-BINDING PROTEINS IN VIRUS-TRANSFORMED CELL LINES.

Melero JA, Salas ML, Salas J, Macpherson IA

Departamento de Biologia Molecular, Instituto B. Maranon, Centro de Investigaciones Biologicas, C.S.I.C., Velazquez, 144, Madrid-6, Spain

J Biol Chem; 250(10):3683-3689 1975

The synthesis of proteins with affinity for DNA was studied in clones of a Syrian hamster cell line (NIL) and subclones of this line transformed by polyoma virus (NIL-Py) or hamster sarcoma virus (NIL-HSV). The synthesis of DNA-binding proteins in NIL and in its virus-transformed derivatives, NIL-Py and NIL-HSV, was very similar in exponentially growing cells, but in dense culture there was a very significant difference in the level of a protein (P8), which was much higher in the transformed lines than in untransformed NIL. Experiments with synchronized cells indicated that the time of maximal P8 synthesis relative to cellular DNA synthesis in NIL-HSV precedes that observed in NIL cells. P8 has a molecular weight of 30,000 as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and is present in large amounts in the transformed cells in dense culture, where it makes up 0.5 to 1% of the total soluble protein. It is concluded that P8 is greatly reduced in dense NIL cells, but is prominant in dense transformed cells. The fact that the high levels in the latter were observed in all the clones of NIL-HSV indicates that this behavior is due to the transformed state, and not to fortuitous clonal selection.

374. DNA-BINDING PROTEINS AND THE G1 TO S TRANSITION IN NORMAL AND VIRUS-TRANSFORMED CELLS.

Macpherson I, Melero JA, Salas M, Salas J

Imperial Cancer Res. Fund Labs., Lincoln's Inn Fields, London, P.O.B. 123, WC2A 3PX England

Biol Haematol; (43):505-522 1976

The NIL-1 cloned line of hamster cells was selected for a study of DNA-binding proteins. Polyoma and hamster sarcoma viruses were used to produce a number of cloned transformed derivatives on agar suspension colonies. The partial revertants obtained from these lines were harvested in the G1 phase of growth stoppage under conditions of medium depletion. The cells were shown to be in the G1 phase by their incorporation of ^{3}H-thymidine after a 10-hr lag, demonstrating their passage through the S phase. At 20 hr after subcultivation, the DNA content per cell was nearly twice that of cells in the stationary phase. The protein profiles

obtained from exponentially growing NIL-1 and NIL-1-HSV cells were similar, and the distribution of peaks resembled that found previously in 3T6 cells. The profiles obtained from dense NIL-1 and NIL-1-HSV cells revealed a striking difference: there was much more P8 protein in the transformed cell extract. A similar result with other cell lines confirmed that this was not due to fortuitous clonal selection. The significance of this 'early' synthesis of P8 in the transformed cell is not clear. (5 refs)

375. DNA-BINDING PROTEINS IN MOUSE CELLS INFECTED WITH POLYOMA VIRUS.

Clertant P, Blangy D
Institut de Recherches Scientifiques sur le Cancer, BP 8, F-94800 Villejuif (France)
Intervirology; 8(1):44-51 1977

DNA-binding proteins from uninfected or polyoma virus-infected mouse cells (3T6) were isolated and compared by DNA-cellulose chromatography and SDSPAGE. During the early phase of infection, few but significant and reproducible differences were observed. In the 0.4 M NaCl eluate, four major new polypeptides were detected in infected cells, with approximate molecular weights of 160 K, 130 K, 86 K and 61 K, the last two being compatible with previous estimates for polyoma T antigen molecular weight. (28 Refs). (Author Abstract)

376. PARTIAL PURIFICATION AND PROPERTIES OF A DNA-BINDING PROTEIN FROM NUCLEI OF CELLS INFECTED WITH POLYOMA VIRUS.

Yeh WS, McGuire M, Center MS, Consigli RA
Div. Biology, Kansas State Univ., Manhattan, Kans. 66506
Biochim Biophys Acta; 418(3):266-276 1976

The partial purification and properties of a DNA-binding protein from polyoma-infected 3T3 cell nuclei are described. The assay used to detect this activity measured the amount of double-stranded DNA retained on a nitrocellulose membrane filter in the presence of binding protein. The interaction between DNA and protein was salt-dependent and occurred optimally at 0.8 M NaCl. The isolated protein could bind to both circular and linear duplex DNA. Incubation of the binding protein with PM2 or polyoma DNA resulted in the formation of a fast sedimenting DNA structure in neutral sucrose gradients. The isolated binding protein was also capable of producing a considerable stimulation of both Escherichia coli (Pol I) and T4 DNA polymerase activities when either single-stranded or intact, native T7 DNA was used as the template. The binding protein itself was free of detectable DNA polymerase or nuclease activity. These results suggest that the stimulatory effect of the binding protein on the polymerases is not due to the presence of nuclease activity which creates a template primer DNA structure.

377. SOLUBILIZATION OF THE EPSTEIN-BARR VIRUS-DETERMINED NUCLEAR ANTIGEN AND ITS CHARACTERIZATION AS A DNA-BINDING PROTEIN.

Luka J, Siegert W, Klein G
Dept of Tumor Biology, Karolinska Inst, S 104 01 Stockholm 60, Sweden
J Virol; 22(1):1-8 1977

The Epstein-Barr virus (EBV)-determined nuclear antigen (EBNA) was solubilized from isolated nuclei of two EBV-transformed cell lines, Raji and AW-Ramos, by high-salt treatment. Its DNA-binding properties were studied by DNA-cellulose chromatography and a ^{51}Cr release complement fixation assay. EBNA binds to both double-stranded and single-stranded calf thymus DNA, showing a higher affinity to double-stranded DNA. There was no detectable difference in the DNA binding of EBNA prepared from Raji and AW-Ramos cells. (40 Refs). (Author Abstract)

378. A COMPARISON OF DNA BINDING PROTEINS FROM NORMAL AND VIRUS-TRANSFORMED MOUSE CELLS.

Rubio V, Tsai WP, Rand K, Long C
Flow Lab., Rockville, Md.
Int J Cancer; 12(3):545-550 1973

The DNA binding proteins present in normal and SV40- or murine sarcoma virus (MSV)-transformed 3T3/B and 3T6 mouse embryo fibroblast cells were analyzed using DNA cellulose chromatography and SDS polyacrylamide gel electrophoresis. Cellular proteins were prelabeled with either 3H or 14C-leucine. Exponentially growing normal mouse 3T6 cells possessed two major DNA binding proteins (P6 and P8) and two minor proteins (P3 and P5) as previously reported. Exponentially growing MSV-transformed 3T6 cells synthesized increased amounts of P3, P5 and P6 compared with normal 3T6 cells, while SV40-transformed 3T3 cells grown under identical conditions had increased levels of P3. Nonproducer 3T3 cells carrying a defective MSV genome and grown under "step-down" conditions of serum depletion produced normal levels of P5, P6, and P8 proteins. These same three proteins were present in decreased amounts in normal 3T3 cells grown under similar conditions.

379. SYNTHESIS OF A DNA BINDING PROTEIN IN NORMAL AND TRANSFORMED CELLS.

Rubio V, Long C
Flow Lab., Inc., Rockville, MD 20852
Int J Cancer; 14(3):348-358 1974

The synthesis of a single-stranded DNA binding protein (P8) by Kirsten sarcoma-virus-transformed and normal mouse cells was measured in serum depleted of growth factors by 3T3/B cells. P8 protein was made by the transformed but not the normal cells and was present during the time of peak DNA synthesis. Normal 3T3/G cells were partially synchronized by growth in depleted serum (and a change to regular medium). Following the medium change, the rate of DNA synthesis increased to a peak between 15 and 20 hr. P8 protein synthesis coincided with the initiation of DNA synthesis and peaked during the same time period.

380. IMMUNOLOGIC STUDIES OF THE LOW MOLECULAR WEIGHT DNA BINDING PROTEIN OF MURINE ONCORNAVIRUSES.

Long CW, Berzinski R, Gilden RV
Viral Oncology Program, NCI-Frederick Cancer Res. Center, P.O. Box B, Frederick, MD 21701
Int J Cancer; 19(6):843-850 1977

A low molecular wt, highly basic DNA-binding protein was purified from several oncornaviruses by the sequential procedures of gel filtration in guanidine-hydrochloride, DEAE-cellulose chromatography and affinity chromatography on single-stranded DNA sepharose. The binding protein from Rauscher and woolly monkey type-C viruses was the fastest migrating of the virion proteins in SDS-polyacrylamide gels and thus is designated p10 according to previous convention although our estimates of molecular wt were 8-9,000 daltons. The binding protein from these two viruses was resolved into two bands by acid-urea electrophoresis although only a single NH_2 terminal amino acid was detected, thus indicating charge heterogeneity. Antibody to Rauscher virus p10 species-specific in gel diffusion and complement-fixation tests did not exhibit cross-reactivity with other virion proteins. A DNA-binding protein was also detected in preparations of mouse mammary tumor virus. This purified protein had an apparent molecular wt of 12,500, was the second fastest migrating component in the virus preparations, and was antigenically unrelated to the mouse type-C virus p10. (Autho abstract) (20 Refs)

381. LOW-MOLECULAR-WEIGHT RAUSCHER LEUKEMIA VIRUS PROTEINS WITH PREFERENTIAL BINDING FOR SINGLE-STRANDED RNA AND DNA.

Davis J, Scherer M, Tsai WP, Long C
Univ. Texas System, Texas Medical Center, Houston, TX 77025

J Virol; 18(2):709-718 1976

A sensitive nitrocellulose filter assay that measures the retention of ^{125}I single-stranded calf thymus DNA was used to detect and purify DNA-binding proteins that retain a biological function from Rauscher murine leukemia virus. By consecutive purification on oligo(dT)-cellulose and DEAE-Bio-Gel columns and centrifugation in 10-30% glycerol gradients, RNA-dependent DNA polymerase was separated from a second virion DNA-binding protein. The binding of this protein to DNA was strongly affected by NaCl concentration but showed little change in activity over a wide range of temperature (15-40 C) or pH (5-10). After glycerol gradient purification, polyacrylamide gel electrophoresis of this protein showed one major band with a molecular weight of approximately 9,800. This protein bound about as well to single-stranded Escherichia coli or calf thymus DNA or 70S type C viral RNA. The binding to ^{125}I single-stranded calf thymus DNA was very efficiently inhibited by unlabeled single-stranded DNA from either E coli or calf thymus and by 70S murine or feline viral RNA. Much larger amounts of double-stranded DNA were required to produce an equivalent percentage of inhibition. This protein, therefore, shows preferential binding to single-stranded DNA or viral RNA.

382. AVIAN TUMOR VIRUS INTERACTIONS WITH CHICKEN FIBROBLAST PLASMA MEMBRANES.

Moldow CF, McGrath M, Van Santen L
Dept. Medicine, Univ. Minnesota Medical Sch., Minneapolis, MN 55455

J Supramol Struct; 4(4):497(457)-506(466) 1976

A method designed to measure the binding of avian tumor viruses to plasma membrane receptors is described. Rous sarcoma virus pseudotypes RSV(RAV-1) and RSV(RAV-2) are purified, and labeled with ^{3}H-uridine and then incubated with gradient-purified and labeled chick embryo fibroblasts (CEF) plasma membranes (CM) (200 ug membrane protein) at 0 C for 25 min. Collected fractions are assayed for viral infectivity in focus forming units (FFU) or acid-precipitable ^{3}H-uridine or ^{3}H-glucosamine. RAV-1 or RAV-2 associates with the membranes and comigrates with them through the gradient to the 40% 10% sucrose interface. With exposure to increasing concentrations of CM, the amount of virus bound also increases. Identical curves are obtained whether the virus position is assayed as infectious FFU or TCA-precipitable ^{3}H-uridine or ^{3}H-D-glucosamine-labeled virus. Virus binding activity is temperature- dependent (between 4 and 37 C) and not affected by prior treatment with ribonuclease A, deoxyribonuclease, or exposure of membranes at 100 C for 5 min. Evidence shows that virus binds to membrane receptors rather than being trapped by membrane fragments. Since genetically resistant C/A and C/B CEF membranes bind both viral subtypes as well as those of sensitive C/O chick cells, the site of initial attachment is not involved in genetic resistance. In addition, RAV-2 binding activity was sensitive to both trypsin and pronase digestion, but RAV-1 was sensitive only to pronase. RAV-2 shows increased binding to membranes obtained from cells with at least 60% membrane neuraminic acid removed by exposure to neuraminidase. Polycation treatment and neuraminic acid removal in CEF both increase virus absorption and infectivity, possibly due to a reduced negative charge at the cell surface. When antireceptor antibody and purified receptor become available, a study of the relationship of virus binding site to cell membrane structure may provide insight into the later stages of penetration. (31 refs)

383. THE GENOME-ASSOCIATED, SPECIFIC RNA BINDING PROTEINS OF AVIAN AND MAMMALIAN TYPE C VIRUSES.

Sen A, Todaro GJ
Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20014

Cell (Camb); 10(1):91-99 1977

A structural protein purified from the Rous sarcoma virus (RSV) can specifically bind in vitro to purified avian, but not mammalian, type C viral RNA. Following ultraviolet irradiation of viral particles under conditions which stabilize the polyploid 70S viral RNA, the same polypeptide can be directly purified from the RSV genome. Based on its electrophoretic mobility in polyacrylamide gels containing sodium dodecylsulfate, the RNA binding protein has been identified as the major phosphoprotein (p19) of avian type C viruses. Similar experiments show that the major phosphoproteins of mammalian type C viruses (p12 for murine viruses and p16 for endogenous primate viruses) are also the specific RNA binding proteins and, similarly, are found closely associated with the 70S RNA genomes in the intact viral particles. (Author Abstract)

384. CHANGES IN SYNTHESIS OF DNA-BINDING PROTEINS DURING THE ONSET OF TRANSFORMATION IN NRK CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS.

Magun BE, Dorsett PH
Dept of Anatomy, Univ of Tennessee Center for the Health Sciences, Memphis, TN 38163.

J Virol; 22(2):469-479 1977

Synthesis of cytoplasmic DNA-binding proteins was investigated after a shift from the nonpermissive to the permissive temperature in NRK cells transformed by a temperature-sensitive mutant of Rous sarcoma virus ts339(RSV). Cells were labeled for several generations in (^{3}H)leucine and were pulse-labeled with (^{35}S)methionine for 1 h at the nonpermissive temperature (39 degrees C) and at the permissive temperature (33 degrees C, 5 h after shift from 39 degrees C). Proteins binding to sequential columns of double-stranded and single-stranded DNA-cellulose were examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and the ^{35}S/^{3}H ratios were obtained for each column fraction and for individual polypeptides. The protein fractions binding to single-stranded, but not double-stranded, DNA and eluting at high salt concentrations (greater than 0.60 M NaCl) showed elevated ^{35}S/^{3}H ratios. This indicated increased synthesis of these proteins within 5 h after the onset of transformation. The majority of the polypeptides in these fractions showed increased synthesis as a consequence of transformation. One prominent polypeptide among them constituted 0.1% of the cytosol protein and had a molecular weight of 93,000. We conclude that the synthesis of proteins binding tightly to single-stranded DNA is increased early after the onset of transformation. (32 Refs). (Author Abstract)

385. SPECIFICITY OF IN VITRO BINDING OF PRIMATE TYPE C VIRAL RNA AND THE HOMOLOGOUS VIRAL P12 CORE PROTEIN.

Sen A, Todaro GJ
Viral Leukemia and Lymphoma Branch, NCI, Bethesda, MD 20014

Science; 193(4250):326-328 1976

The binding of type C viral p12 proteins (molecular weight approx 12,000) to purified viral RNA was studied in vitro using a family of closely related infectious primate type C viruses: the

woolly monkey (SSAV) and the gibbon (GALV) group. The protein-RNA binding was type specific. This remarkable specificity, plus the fact that only a few (less than 25) molecules of protein bind to each RNA molecule in vitro, led to the conclusion that the binding reflects a well-conserved physiologic function. This system could serve as a model for studies of the evolution of nucleic acid binding proteins. (12 Refs)

386. IS THIS THE ORIGINAL CANCER GENE?

Anonymous
No Affiliation Given
New Sci; 69(955):71 1976

The "tumor gene" of the RNA tumor viruses of chickens may have mutated from a normal gene counterpart. This gene, called sarc, upon which the malignant transformation of cells depends, is not related to the previously found endogenous viruses. Its existence was proved in experiments in which labeled DNA representing sarc was matched with sequences in chicken cells from which all non-sarc sequences had been removed. These sequences are not unique to chickens but are present in many other birds. As genetic distance from the chicken increases, the sarc DNA sequence becomes increasingly obscure. The original gene possibly functioned in both normal embryonic development programming and non-viral cancers. As further evidence of its existence, the quail sarc-like sequence is transcribed into RNA in a chemically induced tumor. However, there are several RNA viruses that do not cause solid tumors and do not carry sarc but can still transform cells in culture. Nor has any sarc-like gene been identified in the DNA tumor viruses, which apparently transform cells via a DNA-binding protein.

B. Other Specific Functions and Properties of DNA and RNA Binding Proteins

387. A CLINICAL EVALUATION OF SERUM C3DP LEVELS IN INDIVIDUALS WITH MALIGNANT DISEASES.

Parsons RG, Longmire RL, Hoch SO, Hoch JA
Departments of Microbiology, Scripps Clinic and Research Foundation, La Jolla, California 92037
Cancer Res; 37(3):692-695 1977

A human serum DNA-binding protein (C3DP) derived from complement component C3 has been found in elevated concentrations in the sera of individuals with malignant diseases. An assay system has been devised which reveals quantitative values of serum C3DP levels. Results obtained using this system indicate that normal human sera have an average C3DP level of 83 ug/ml (range, 40 to 146), whereas sera from individuals with active carcinomas have an average C3DP level of 242 ug/ml (range, 146 to 400). Sera from individuals with active leukemias, lymphomas, and melanomas all had elevated levels of C3DP, whereas sera from individuals with polycythemia vera or other nonmalignant diseases had normal or only slightly elevated C3DP concentrations. No tissue specificity seems to be required for malignant growths to result in elevated C3DP serum concentrations. The levels of C3DP in 79% of the individuals who experienced disease remission were found to decline to normal values, concurrent with the disease regression. Patients who did not respond to therapy regimens retained high C3DP levels. (Author Abstract)

388. DNA-BINDING PROTEINS IN YOUNG AND SENESCENT NORMAL HUMAN FIBROBLASTS.

Stein GH
Stanford Univ. Sch. Med., Calif.
Exp Cell Res; 90(2):237-248 1975

The relationship of DNA binding proteins (DBP) to the replicative state in young and senescent WI38 human fibroblast cells was examined to determine if DBP play a role in the regulation of cell replication. The amounts of DBP in different cell populations were measured by adding radioactive amino acid precursors to the cells 40 hours before isolation of the DBP using DNA-cellulose chromatography and polyacrylamide gel electrophoresis. Five proteins, P5b (87,000 daltons), P6a (50,000), P5 (33,000), P9 (28,000) and P10 (25,000) were labeled more in replicating than in stationary cells. Two proteins, P5c (72,000 daltons) and P12 (18,000) were labeled more in the stationary phase cells. Several DBP of high molecular weight were labeled to a greater extent in stationary phase cells, and these were partially characterized as protocollagen and collagen. Senescent WI38 cells, near or at the end of their in vitro lifespan, showed increased proportions of P8 in the stationary phase when compared to WI38 cells at early doubling levels. In the growth phase, P8 amounts to more than 1% of the total soluble protein in young cells, and binds preferentially to single stranded DNA. It is concluded that WI38-P8 is comparable to a previously-characterized P8 from human SB and mouse 3T6 cells. It is suggested that increased amounts of P8 in senescent cells may be involved in the irreversible inhibition of replication at the end of the in vitro lifespan.

389. ANTI-DNA ANTIBODIES IN PATIENTS WITH LEUKEMIA AND LYMPHOMA.

Izui S, Lambert PH, Carpentier NA, Miescher PA
WHO Immunology Research and Training Centre, Centre de Transfusion, Hopital Cantonal, CH-1211 Geneva, Switzerland
Schweiz Med Wochenschr; 106(40):1377-1378 1976

Samples of sera (217) from 33 patients with acute myeloid leukemia (AML), 42 with chronic myeloid leukemia (CML), 12 with acute lymphatic leukemia (ALL), 22 with lymphoma, and 20 with other malignant diseases were examined radioimmunologically for the presence of antibodies against DNA. The levels of ^{125}I-labeled single-strand DNA (ssDNA) were significantly higher in all three types of leukemia and lymphoma compared to control: 70% of AML, 50% of CML, 40% of ALL, and 30% of lymphoma sera showed the presence of anti-ssDNA antibodies. However, none exhibited a positive reaction with double-stranded DNA. ^{125}I-ssDNA binding activity was also measured by a double antibody method. Most of the sera from leukemic patients showed a lower but still significant level of binding activity to ssDNA, confirming that the DNA-binding proteins were immunoglobulins. In some serum samples the DNA may be bound nonspecifically to nonimmunoglobulin basic proteins, leading to false-positive results in the Farr assay. Induction of anti-DNA antibodies is independent of the drugs and vaccinations used in therapy. Serial studies on sera from several CML patients showed that in some cases the level of serum anti-DNA antibodies increased after the decrease of WBC number. Anti-DNA antibodies may be involved in the formation of DNA-anti-DNA complexes that can lead to vascular or glomerular lesions and also possibly interfere with the effector mechanisms of cell-mediated immunity against leukemia surface antigens, particularly at the level of antibody-dependent cell-mediated cytotoxicity. However, the possibility that such anti-DNA antibodies could be induced by a viral agent cannot be excluded. (3 refs)

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390. USE OF DNA COLUMNS TO SEPARATE AND CHARACTERIZE NUCLEAR NONHISTONE PROTEINS.

Allfrey VG, Johnson EM

Chromosomal Proteins and Their Role in the Regulation of Gene Expression; Stein, G. S.; Kleinsmith, L. J., eds. (New York: Academic Press, Inc.): pp 265-300; 1975

New techniques of DNA affinity chromotography are which aid in the study of DNA binding properties and functions of nuclear nonhistone proteins. Methods were developed for the covalent attachment of single and double stranded DNA molecules to solid supports, such as, Sepharose 4B, aminoethyl-Sepharose 4B, Sephadex G-25, and Sephadex G-200. The DNA bound proteins are displaced by stepwise increments in the salt concentration of the eluting buffer to yield sets of proteins, which differ in complexity and size distribution. Highly reproducible patterns elution of nuclear nonhistone proteins are obtained from replicate DNA columns, and the proteins eluted at a given salt concentration emerge at the same salt concentration on rechromatography. Comparisons of the elution profiles and electrophoretic patterns of nuclear nonhistone proteins eluted from parallel columns containing low Cot, intermediate cot, and high Cot DNA sequences reveal that some of the proteins interact preferentially with particular DNA sequences. Several nucleotide binding proteins are released when the DNA columns are treated with deoxyribonuclease. A highly purified histone H1 kinase binds specifically to the DNA of the species of origin. DNA binding resides in the catalytic, rather than the regulatory, subunit of this enzyme. Studies of the composition and metabolism of lymphocyte nuclear nonhistone proteins in cells stimulated by Concanavalin A show that many of the proteins enter the nucleus from the cytoplasm shortly after exposure to the mitogen. This intracellular movement appears to be highly selective, in the sense the not all nuclear proteins increase proportionately during gene activation. (38 refs)

391. SUBCELLULAR DISTRIBUTION OF DNA-BINDING PROTEINS FROM CULTURED HAMSTER FIBROBLASTS.

Melero JA, Salas J, Salas ML

Centro de Biologia Molecular, C.S.I.C.-U.A.M.Velazquez 144, Madrid-6, Spain

Biochim Biophys Acta; 437(2):462-476 1976

The distribution between the nuclei and cytoplasm of DNA-binding proteins from growing NIL hamster sarcoma cells was studied. To obtain the subcellular fractions, cell monolayers or cells previously detached from the culture dish were treated with the nonionic detergent Nonidet P-40. Proteins with affinity for DNA were isolated from nuclear or cytoplasmic fractions by chromatography on DNA-cellulose columns and were further analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. P8, one of the major components in the 0.15 M NaCl-eluted proteins, was found predominantly in the cytoplasmic fractions, whereas P6, the other main protein peak in this eluate, was more prominent in the nuclear fraction. Among the other proteins, P5 and P5' were detected in both the nuclear and cytoplasmic fractions. All the others were present almost exclusively in the cytoplasmic fraction. In contrast, most of the proteins with higher affinity for DNA, eluted from the column at 2 M NaCl, were present in the nuclear fraction, although they were also detected in the cytoplasm in amounts similar to those observed in the nuclei. (23 refs)

392. SOME PROPERTIES OF A DNA-UNWINDING PROTEIN UNIQUE TO LYMPHOCYTES FROM CHRONIC LYMPHOCYTIC LEUKEMIA

Huang AT, Riddle MM, Koons LS

Duke Univ. Med. Cent., Durham, N.C.

Cancer Res; 35(4):981-986 1975

Lymphocytes from a common human leukemia, chronic lymphocytic leukemia, have a greatly enhanced capability of DNA repair and a concomitantly prolonged survival in vitro after damage to DNA. A DNA-binding protein with a molecular wt of 24,000 was isolated and purified from lymphocytes of four patients with chronic lymphocytic leukemia. The purification process involved passing crude cell homogenates twice through a double-stranded DNA column and rechromatographing the flow-through in a UV-irradiated DNA cellulose column. The protein binds tightly to both UV-irradiated and single-stranded DNA. At 35 C it enhances the helix-coil transition of polycd(A-T) and the UV-irradiated calf thymus DNA but is inefficient in ordinary native DNA. This protein also facilitates the rate of UV-endonuclease incision of UV DNA but does not induce any nicks by itself. This finding suggests that the protein may be involved in DNA repair by enhancing such activity, and also offers an explanation for the increased DNA repair observed in chronic lymphocytic leukemia cells. When human metaphase chromosomes are exposed to the protein, it induces marked lengthening of chromatids suggesting that this protein may also act on complex chromosomes. By quantitative immunochemical determinations, such protein could not be found in lymphocyte extracts of three normal individuals.

393. PURIFICATION AND PHYSICAL CHARACTERIZATION OF NUCLEIC ACID HELIX-UNWINDING PROTEINS FROM CALF THYMUS.

Herrick G, Alberts B

Department of Biochemical Sciences, Princeton University,Princeton, New Jersey 08540

J Biol Chem; 251(7):2124-2132 1976

We have devised a general protein fractionation procedure which selects for eukaryotic DNA-binding proteins, some of which resemble DNA-unwinding proteins from prokaryotes. Proteins were selected which (a) pass through a native DNA-cellulose column, (b) bind to a denatured DNA-cellulose column, and (c) remain bound to the latter column during a rinse with a dilute solution of the sodium salt of the polyanion dextran sulfate. When this fractionation was applied to the soluble proteins of calf thymus, three major protein species were recovered. The predominant one has an apparent molecular weight of about 24,000 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, is isoelectric near neutrality, and elutes as a monomer from denatured DNA-cellulose at moderate NaCl concentrations. This protein, designated calf-unwinding protein 1 (UP1), has been purified to homogeneity. However isoelectric focusing reveals four or five subspecies (apparently separated by single-charge differences) which differ appreciably in their affinities for DNA. Two other major proteins are obtained which have apparent molecular weights in sodium dodecyl sulfate of 33,000: the first, which elutes with low salt from DNA-cellulose as a nomogeneous preparation, appears to be a basic protein (although it is clearly not a histone); the other, which elutes from DNA-cellulose as the major component of a "high salt eluting fraction," is an acidic protein which co-purifies with less prominent species of higher molecular weights. Proteins similar to each of these three major calf thymus proteins have been observed by us and others in tissue culture cells of mouse, hamster, monkey, and humans, suggesting their wide occurrence among eukaryotes.

394. DNA-BINDING PROTEINS RELATED TO THE DOSAGE OF SPECIFIC YEAST CHROMOSOMES.

Finkelstein DB, Butow RA
Southwestern Medical Sch., Dallas, Tex. 75235
Biochem Biophys Res Commun; 66(4):1365-1372 1975

A comparison was made of the in vitro DNA-binding proteins of specific aneuploid and isogenic euploid cells of Saccharomyces cerevisiae by DNA-cellulose chromatography. Changes in the level of a small fraction of the yeast DNA-binding proteins that could be related to the dosage of specific yeast chromosomes were detected. At least 4 proteins showed a dosage related to the cellular level of chromosome I, and at least 1 protein showed a dosage related to the level of chromosome VI.

395. DNA-BINDING PROTEINS IN YEAST. EFFECT OF GROWTH PHASE AND MITOCHONDRIAL FUNCTION.

Finkelstein DB, Butow RA
Univ. Texas Southwestern Medical Sch., Dallas, TX 75235
Arch Biochem Biophys; 174(1):52-65 1976

DNA-binding proteins of the yeast Saccharomyces cerevisiae were examined by DNA-cellulose chromatography. After a high speed supernatant of a deoxyribonuclease-treated cell lysate was passed through a column of calf thymus DNA-cellulose, the DNA-binding proteins were eluted with a discontinuous salt gradient. The DNA-binding proteins, which showed a broad distribution in size when examined by electrophoresis on polyacrylamide slab-gels in the presence of sodium dodecyl sulfate, represented about 0.2-0.3% of the cell's protein, corresponding to about 5 x 10^6 -molecular weight of protein per haploid cell. Quantitative and qualitative changes in the spectrum of DNA-binding proteins were correlated with changes in growth rate, stage of the growth cycle, and phenotypic (repressed vs derepressed) and genetic alterations in mitochondrial function (grandes vs petites). The largest change in the spectrum of DNA-binding proteins was between glucose-grown log-phase grande cells and grande cells in stationary phase. A number of specific DNA-binding proteins varied by as much as 5-10 x. From estimates of the number of molecules of a DNA-binding protein present in the cell, it is concluded that the system described is capable of detecting less than 100 molecules per yeast cell within the range of the level of the lac repressor in Escherichia coli.

396. THE EFFECT OF 5-BROMODEOXYURIDINE ON THE SYNTHESIS OF NUCLEAR AND CYTOPLASMIC DNA-BINDING PROTEINS ISOLATED FROM SEA URCHIN EMBRYOS.

Case ST, Talkington CA, Baker RF
Dept. Biol. Sci., Univ. Southern California, Los Angeles CA 90007
Cell Differ; 4(1):55-62 1975

DNA-binding proteins synthesized by morula stage sea urchin embryos (Strongylocentrotus purpuratus) growing in the presence of 5-bromodeoxyuridine (BUdR; 50 microg/ml) were studied. Continuous exposure to BUdR from fertilization through cleavage did not affect the gross rates of synthesis of nuclear or cytoplasmic DNA-binding proteins synthesized in morula stage embryos. Data shows that proteins with molecular wt greater than histones are being synthesized. Essentially identical results were obtained with blastula stage embryos. Failure to detect any BUdR-induced variations in the synthesis of DNA-binding proteins at the morula stage is not the result of a high proportion of histone synthesis or of radio-labeled DNA-binding proteins that have been translated from stored maternal mRNA. Continuous exposure to BUdR does not effect translation, during embryogenesis, of mRNA synthesized and stored during oogenesis and coding for the DNA-binding proteins analyzed in this study.

397. ASSOCIATION OF THETAX174 DNA-DEPENDENT ATPASE ACTIVITY WITH AN ESCHERICHIA COLI PROTEIN, REPLICATION FACTOR Y, REQUIRED FOR IN VITRO SYNTHESIS OF THETAX174 DNA.

Wickner S, Hurwitz J
Viral Carcinogenesis Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014
Proc Natl Acad Sci; 72(9):3342-3346 1975

ThetaX174 DNA-dependent DNA synthesis is catalyzed in vitro by the combination of at least 11 purified protein fractions: dnaB, dnaC(D), and dnaG gene products, DNA polymerase III, DNA elongation factors I and II, DNA binding protein, and replication factors W, X, Y, and Z. The reaction requires ATP, 4 dNTPs, and Mg**+2 and is specific for ThetaX174 (or ThetaXahb) DNA. Purified replication factor Y contains ThetaX174 (or ThetaXahb) DNA-dependent ATPase (for dATP-ase) activity. The ATPase activity is poorly stimulated by other single-stranded DNA, by double-stranded DNA, or by RNA. The products of the ThetaX174 DNA-dependent ATPase activity of factor Y are Pi and ADP (or dADP). The association of ThetaX174 DNA-dependent ATPase activity with factor Y was shown in the following ways: (a) the two activities copurified with a constant ratio; (b) they comigrated on native polyacrylamide gel electrophoresis; (c) both activities were heat-inactivated at the same rate; and (d) both showed identical patterns of N-ethylmaleimide sensitivity. (Author Abstract)

398. SELECTIVE INHIBITION OF IN VITRO DNA SYNTHESIS DEPENDENT ON PHIX174 COMPARED WITH FD DNA. I. PROTEIN REQUIREMENTS FOR SELECTIVE INHIBITION.

Vicuna R, Hurwitz J, Wallace S, Girard M
Dept of Developmental Biology and Cancer, Div of Biological Sciences, Albert Einstein Coll of Medicine, Bronx, NY 10461
J Biol Chem; 252(8):2524-2533 1977

Crude extracts of Escherichia coli selectively convert fd viral DNA and not phiX174 DNA to duplex DNA via a complex series of reactions one of which involves RNA polymerase. Reactions leading to formation of fd duplex-replicative (RFII) structures have been reconstituted with purified proteins from E coli. Maximal synthesis requires the combined action of E coli binding protein, DNA elongation factor I, DNA elongation factor II preparations (which are a mixture of dna Z and DNA elongation factor III), DNA polymerase III, DNA-dependent RNA polymerase, Mg^{2+}, dATP, dGTP, dCTP, dTTP, and ATP, GTP, CTP, and UTP. In contrast to crude extracts of E coli, purified protein fractions do not distinguish between fd DNA and phiX174 DNA in duplex DNA formation. The addition of crude fractions of E coli to the purified components listed above selectively permits fd RFII formation and prevents phiX RFII formation. This selective inhibition was used as an assay to isolate proteins essential for this phenomenon; they include RNase H, discriminatory factor alpha, and discriminatory factor beta. (35 Refs) (Author Abstract)

399. SELECTIVE INHIBITION OF PHIX RFII COMPARED WITH FD RFII DNA SYNTHESIS IN VITRO. II. RESOLUTION OF DISCRIMINATION REACTION INTO MULTIPLE STEPS.

Vicuna R, Ikeda J, Hurwitz J
Dept of Developmental Biology and Cancer, Div of Biological Science, Albert Einstein Coll of Medicine, Bronx, NY 10461
J Biol Chem; 252(8):2534-2544 1977

In the presence of RNA polymerase, RNase H, discriminatory factors alpha and beta, Escherichia coli binding

protein, DNA elongation factor I, DNA elongation factor II preparation, DNA polymerase III, and ATP, UTP, GTP, CTP, dATP, dTTP, dGTP, and dCTP, fd viral DNA can be quantitatively converted to RFII containing a unique gap in the linear minus strand. This gap, mapped with the aid of restriction endonucleases HinII and HpaII, is located within Fragment Hpa-H of the fd genome. The discrimination reaction has been resolved into two steps: Step A, fd viral DNA, E coli binding protein, and discriminatory factors alpha and beta form a protein DNA complex; Step B, the complex isolated by agarose gel filtration selectively forms fd RFII when supplemented with RNase H, RNA polymerase, and the DNA elongation proteins. The omission of any of the proteins described above during the first reaction resulted in either no discrimination or a decrease in discrimination when the missing protein was added during the second step. Results are presented which indicate that E coli binding protein, discriminatory factors alpha and beta, and RNase H must be present during the time RNA synthesis occurs in order to selectively form RFII from fd DNA and not thetaX RFII. The amount of fd and phiX174 RNA-DNA hybrid formed in vitro is directly related to the DNA synthesis observed. Thus, under discriminatory conditions, only fd viral DNA leads to fd RNA-DNA complexes and no phiX RNA-DNA hybrid is formed. Under nondiscriminatory conditions, both DNAs yield RNA-DNA hybrids and DNA synthesis. In the absence of discriminatory factor alpha, no RNA-DNA hybrid is formed with either DNA, and in turn, no DNA synthesis is detected with either DNA template. (22 Refs) (Author Abstract)

400. SELECTIVE INHIBITION OF THE DNASE ACTIVITY OF THE RECBC ENZYME BY THE DNA BINDING PROTEIN FROM ESCHERICHIA COLI.

Mackay V, Linn S
Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey 08903
J Biol Chem; 251(12):3716-3719 1976

In the presence of the Escherichia coli DNA binding protein, single-stranded DNA is resistant to both the endo- and exonucleolytic activities of the recBC DNase. Linear duplex DNA, on the other hand, is unwound at a normal rate, but converted to large, single-stranded fragments which are resistant to further hydrolysis. Therefore, in the presence of the binding protein and linear duplex DNA, the recBC enzyme acts not as a DNase, but primarily as an ATP-dependent unwinding enzyme, able to generate large, single-stranded material. Duplex circular DNA containing short, single-stranded gaps is also resistant to the hydrolysis in the presence of the binding protein. (Author Abstract)

401. DNA BINDING PROTEINS EXTRACTED FROM RAT LIVER NUCLEI BY LOW IONIC STRENGTH BUFFERS.

Prestayko AW, Schmoll DJ, Ganpath N, Goldknopf I, Busch H
Dept. Pharmacology, Baylor Coll. Med., Houston, Tx. 77025
Proc Am Assoc Cancer Res; 16:53 1975

Nonhistone chromatin proteins implicated in gene regulation include proteins soluble in low ionic strength buffers. Rat liver nuclei were extracted twice with 0.01 M Tris-HCl, pH 7.0, 0.15 M NaCl, 0.001 M MgCl2, 0.001 M PMSF and 4 times with the same buffer at pH 8.0. Chromatin was extracted with 0.35 M NaCl, 0.01 M Tris-HCl, pH 8.0, 0.001 M PMSF. DNA polyacrylamide columns were used to study binding of the nuclear proteins to rat liver and Novikoff hepatoma DNA. Approximately 7-13% of the protein applied was bound to DNA in the column. The 2D gel electrophoretic patterns of the proteins eluted from the columns with 2 M KCl were similar for tumor and normal rat liver DNA. Two proteins (As,Ar) of 30,000 MW were quantitatively retained on the column. Other proteins of higher molecular weight including Bp, BC, B22, B24, CM, CN and CQ were bound to the DNA in relatively high amounts. Of these, B24 and Bp comprise the major proteins of the nuclear 30S informosomal particle. Phosphoproteins which had been labeled in vivo for 2 hours with ^{32}Pi were separated on 2D gels and subjected to autoradiography. Approximately 10 proteins were labeled. With the possible exception of 1 or 2 spots, the labeled proteins were different from those which bind to DNA. These results suggest that the DNA binding capacity and phosphorylation of proteins are unrelated. (Author Abstract)

402. A DNA BINDING PROTEIN FROM HUMAN PLACENTA SPECIFIC FOR ULTRAVIOLET DAMAGED DNA.

Feldberg RS, Grossman L
Dept. Biochemistry, Brandeis Univ., Waltham, MA 02154
Biochemistry (Washington); 15(11):2402-2408 1976

A DNA-binding protein specific for UV-irradiated DNA was purified extensively from human placenta. The binding preparation was free of exonuclease, polymerase, endonuclease, and N-glycosidase activity. The binding activity was salt-dependent and specific for double-stranded irradiated DNA. DNA from which the pyrimidine dimers were monomerized by the action of photolyase (photoreactivating enzyme) remained an effective substrate for the binding protein, suggesting that the protein recognizes photoproducts other than pyrimidine dimers. This is supported by the finding that DNA irradiated under conditions that introduce only pyrimidine dimers was not a substrate for the binding protein. Examination of three of the xeroderma pigmentosum complementation groups revealed no deficiency in this binding activity. (29 refs)

403. IN VITRO STUDIES OF PHOTOCHEMICALLY CROSS-LINKED PROTEIN-NUCLEIC ACID COMPLEXES. DETERMINATION OF CROSS-LINKED REGIONS AND STRUCTURAL RELATIONSHIPS IN SPECIFIC COMPLEXES. (PP. 123-148)

Schimmel PR, Budzik GP, Lam SS, Schoemaker HJ
Aging, Carcinogenesis, and Radiation Biology. The Role of Nucleic Acid Addition Reaction. Smith KC, ed. New York, Plenum Press, 1976.

The in vitro coupling of specific proteins to nucleic acids is reviewed. Various photoinduced covalent complexes are considered, including protein-DNA complexes such as (BSA)-DNA, DNA polymerase-DNA, RNA polymerase-DNA, lac repressor-DNA, and bacteriophage fd gene 5 protein-DNA. The specificity of cross-linking and the analysis for cross-linked regions on tRNA are discussed in relation to the coupling of tRNAs to aminoacyl tRNA synthetases. The variety of systems in which successful cross-linking has been accomplished suggests that nucleic acid binding proteins, may typically be capable of photo-joining to their appropriate nucleic acids. This approach has considerable value for structure-function studies. Together with other structural information on individual reacting macromolecules, the cross-linking and sequence information may be sufficient to build a well-defined molecular model of a complex of interest.

404. PHOTOINDUCED CROSS-LINKAGE, IN SITU, OF ESCHERICHIA COLI 30S RIBOSOMAL PROTEINS TO 16S RRNA: IDENTIFICATION OF CROSS-LINKED PROTEINS AND RELATIONSHIPS BETWEEN REACTIVITY AND RIBOSOME STRUCTURE.

Gorelic L

Dept. Chemistry, Wayne State Univ., Detroit, MI 48202

Biochemistry (Washington); 15(16):3579-3590 1976

The kinetics of photoinduced cross-linkage of Escherichia coli 30S ribosomal proteins to the 16S rRNA molecule in the intact E coli 30S ribosomal subunit were studied. All 30S ribosomal proteins became cross-linked to the 16S rRNA before changes in the sedimentation characteristics of the 30S ribosomal subunit could be detected. The proteins exhibited different reactivities in the cross-linkage reaction. One group of proteins - S3, S7-S9, S11, S12, and S15-S19 - was cross-linked to the 16S rRNA by single-hit kinetics, or by photoprocesses of nonunity but low multiplicities. A second group of proteins - S1, S2, S4-S6, S10, S13, S14, and S21 - was cross-linked to the 16S rRNA by photoprocesses of a complex nature. A comparison of these data with other properties of the individual 30S ribosomal proteins, related to ribosome structure, indicated that most of the 30S ribosomal proteins cross-linked to the 16S rRNA by photoprocesses of low multiplicities had been classified rRNA-binding proteins by nonphotochemical methods, and that most of the proteins cross-linked to the 16S rRNA by photoprocesses of large multiplicities had been classified as non-binding proteins. There were certain exceptions to these correlations. Proteins S4 and S20, both RNA-binding proteins, became cross-linked to the 16S rRNA by photoprocesses of large multiplicities, and proteins S3, S11, S12, and S18, none of which have been classified RNA-binding proteins, exhibited low multiplicities in the cross-linkage reaction. All exceptions could be explained in terms of limitations inherent in the photochemical methods used in this study and in other types of methods that have been used to study RNA-protein interactions in the 30S ribosomal subunit. The data also suggested that labile RNA-protein cross-links are present in the UV-irradiated 30S ribosomal subunits, and that neither peptide-bond cleavage nor photoinduced modification of the charged side-chain groups in the ribosomal proteins accompanied the cross-linkage reaction. Some photoinduced RNA-chain breakage might have occurred. These results suggest that the E coli ribosomal subunits might be useful as model systems for establishing protocols to be used in the application of photochemical methods to studies of nucleic acid-protein interactions in other large nucleoprotein complexes.

405. PROPERTIES OF A SURFACE RECEPTOR FOR POLY(U) ON 13762 RAT MAMMARY ADENOCARCINOMA ASCITES CELLS (MEETING ABSTRACT).

Kocemba-Sliwowska U, Fairbanks G

Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

J Cell Biol; 70(2/Part2):240a 1976

3H-poly(U) are used as a probe of surface structure on ascites cells of the 13762 rat mammary adenocarcinoma. Binding of 3H-poly(U) is complete within three minutes at O C and exhibits specificity for the ribonucleoside moiety of the polymer. The fraction of nonviable cells is not correlated with the magnitude of binding, which is reproducibly 3 x 10 uridine residues (about 10 chains) per cell at O C in Hank's balanced salt solution. Cells preincubated for five minutes at 37 C with trypsin, chymotrypsin pronase or papain at 5 ug/ml suffered reductions of up to 72% in their capacity to bind poly(U). Treatment with 5% glutaraldehyde also sharply reduced binding capacity. Minimal reductions (2-11 2%) resulted from pretreatment with neuraminidase, deoxyribonuclease or ribonuclease T1, and pre-incubation with concanavalin A (37 C) or metabolic inhibitors had no effect. Although a substantial fraction of the bound 3H-poly(U) could be displaced by uridine and other ribonucleosides, treatment of the polymer-cell complex with proteases and other hydrolytic enzymes released less than 6% of the radioactivity. Ribonuclease A extensively degraded cell-associated 3H-poly(U) (based on characterization of phenol-extracted radioactivity), but the fragments produced were not released to the medium. These and other results suggest that each bound poly(U) chain engages in multiple interactions with arrays of protein receptors at the cell surface.

V. CARCINOGENESIS AND CARCINOGEN BINDING PROTEINS

A. Azodye Binding Proteins

406. EVIDENCE FOR A PROTEIN RECEPTOR OF ACTIVATED AZOCARCINOGEN. (MEETING ABSTRACT).

Mainigi KD, Sorof S

Inst for Cancer Res, Philadelphia, Pa. 19111

Proc Am Assoc Cancer Res; 18:78 1977

During carcinogenesis in rat liver by 3'-methyl-4-dimethylaminoazobenzene, the two moieties of the principal liver carcinogen-protein complex (h2-5S azoprotein) have different turnover rates. The half life of the bound dye is 2.5 plus or minus 0.25 days, while that of protein is 8.7 plus or minus 1.6 days (probability of identity less than 0.001). This difference suggests that the two moieties of the complex are non-covalently linked, and have different biological activities. The native complex apparently contains activated azo dye, since after protein denaturation the two moieties appear to be covalently linked. Interaction with azocarcinogen in vivo extends the half life of the protein moiety from 3.3 plus or minus 0.2 days to 8.7 days. This retardation in turnover agrees with the stabilization in vitro and slower proteolysis in vivo of other proteins caused by specific ligands, due to more compact configuration. The carcinogen-protein interaction appears to be reversible, since cessation of carcinogen feeding soon reduces the protein's half life to 3.4 plus or minus 0.2 days, as in normal liver. Our data are explainable by a model in which the principal carcinogen-protein complex contains hydrophobically bound activated carcinogen, whose specificity of reaction with critical macromolecule(s) in cell nuclei may be directed by the conformationally altered protein of the complex, ie, a specific receptor protein containing activated azocarcinogen. (Author Abstract)

407. DISTRIBUTION OF BASIC AZO-DYE-BINDING PROTEIN IN NORMAL RAT TISSUES AND CARCINOGEN-INDUCED LIVER TUMORS.

Bannikov GA, Guelstein VI, Tchipysheva TA

Inst. Exp. Clin. Oncology AMS, USSR

Int J Cancer; 11(2):398-411 1973

Monospecific antibodies against basic azo-dye-binding protein from the rat liver were obtained. An antigen identical to this protein was shown to be present in the liver, kidney, small intestine, ovary and testicle extracts of rats and also in liver and kidney extracts of mice. The concentration of this antigen was significantly reduced in the liver and kidney of rat embryos and in rats during the early postnatal period. Immunofluorescence technique was employed to study

distribution of the azo-dye-binding protein in sections of normal rat liver and liver tumors induced by 3'-methyl-4-dimethylaminoazobenzene). This protein was found in the hepatocytes of the central areas of the acinus but not in the hepatocytes of periportal zones. The azo-dye-binding protein was absent from poorly-differentiated hepatomas, anaplastic carcinomas and from most adenocarcinomas. In all the highly-differentiated hepatomas this protein was found to be uniformly distributed among the tumor cells. Thus the presence of azo-dye-binding protein in tumors seems to depend on the degree of cell differentiation.

408. CARCINOGEN-BINDING PROTEIN DISTRIBUTION IN PRIMARY LIVER TUMORS INDUCED IN RATS BY 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE.

Bannikov GA, Gel'shteyn VI, Chipysheva TA
Inst. Exp. Clin. Oncol., Moscow, USSR
Biull Eksp Biol Med; 75(3):90-92 1973

Primary liver tumors were induced in Wistar rats by 3'-methyl-4-dimethylaminoazobenzene (0.6 g/kg food). Multiple liver tumors, which developed in rats fed this diet for 4.5 months, included 4 highly differentiated trabecular hepatomas; 13 mixed tumors consisting of adenocarcinomas, poorly differentiated hepatomas and anaplastic carcinomas; and 2 anaplastic carcinomas. Sections from these 19 primary liver tumors were examined by an immunofluorescent method, using monospecific antibodies to the carcinogen-binding protein. The level of this protein was directly related to the degree of tumor cell differentiation. The carcinogen-binding protein was absent in poorly differentiated hepatomas, anaplastic carcinomas, and adenocarcinomas but was present in highly differentiated hepatomas, in which it was evenly distributed in all cell populations.

409. IRREVERSIBLE CHANGE OF THE PATTERN OF CARCINOGENIC AMINOAZO DYE-BINDING PROTEINS IN RAT LIVER DURING CONTINUOUS FEEDING OF 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE.

Sugimoto T, Terayama H
Fac. Sci., U. Tokyo, Japan
Cancer Res; 31(10):1478-1482 1971

The permanence of changes in 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB), binding proteins isolated from heat-treated cell sap of rat hepatocytes was studied. Rats fed a dye-containing diet for either 2 wks or one mo and then maintained on a dye-free diet were given an intragastric dose of 40 mg 3'-Me-DAB. The dye-binding proteins were separated by carboxy-methylcellulose chromatography or Sephadex G-100 gel filtration into several fractions, with fractions I, IV, VI and VII containing the dye-protein complexes. The ratio of binding to two components of Fraction I, Ia and Ib, changes with continuous dye feeding and relatively more dye was bound to Ib. The subsequent 3'-Me-DAB-free period did not alter the overall binding capacity of fraction I nor did the relative binding capacity of the components change. This suggests that the changes in binding of 3'-Me-DAB were permanent. Similar changes were noted in components IV-a, IV-b and IV-c of fraction IV. Controls had high binding in IV-a. With continuous dye feeding, however, binding to this component decreased and binding increased greatly in IV-c. Since permanent binding changes were seen in the acidic protein fractions I and IV, it was postulated that these changes may be involved in mechanisms of carcinogenesis.

410. INTERACTIONS OF AZODYE CARCINOGENS AND AZODYE CARCINOGEN CONJUGATES WITH SPECIFIC PROTEINS IN THE RAT LIVER.

Ketterer B, Beale D, Litwack G, Hackney JF
Middlesex Hosp. Med. Sch., London, England
Chem Biol Interact; 3:285-286 1971

Some physical and chemical properties of azodye-(ie, N, N-dimethyl-4-aminoazobenzene and derivatives) binding proteins of the soluble cytoplasm of the liver were described. Apparently, some of the bound azodye is in the fact strongly bound azodye conjugate. The azodye-binding proteins include a basic protein and low molecular wt protein. The basic protein has been shown to bind metabolites of the skin carcinogen 3- methylcholanthrene. The molecular wt of the basic protein is 45000, and that of the low molecular wt protein is 14000. In the basic protein, the amino acid residue which binds the azodye is CyS; amino acid analysis of the performic acid-oxidized azodye conjugate reveals that its constituents are Glu, CySO3H and Gly. The amino acid which binds the azodye in the low molecular wt protein is Met.

411. THE CHANGING PATTERN OF CARCINOGENIC AMINOAZO DYE-BINDING PROTEINS DURING THE COURSE OF CONTINUOUS FEEDING OF 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE.

Sugimoto T, Terayama H
Fac. Science, U. Tokyo, Japan
Chem Biol Interact; 2(4):391-400 1970

Male Wistar rats fed a semisynthetic diet containing 0.06% 3'-methyl-4-dimethylaminoazobenzene and water ad lib were sacrificed at 2 wk, 1, 3 or 10 months, and the livers were homogenized to provide a liver sap for protein-bound dye and arginase assay. Protein content of the cell sap ranged from 17.4-27.1 mg/g liver; protein-bound dye range from 2.4-15.4 nmoles/g liver. The arginase content ranged from 164-346 U/g liver in azo dye fed animals compared to 5 control rats with 143 U/g and 3 hepatoma-bearing rats with 29 U/g. Throughout the study the protein-bound dye as well as the arginase activity present in the liver cell sap was recovered almost completely in response to heat-treatment; the enzyme activity in livers of rats on continuous-azo dye feeding proved to be rather labile, compared with activity noted in previous experiments with single dosages, and was ultimately lost with subsequent chromatographic separation. As dye feeding progressed an increasingly retarded elution pattern for basic protein fractions which was associated with more and more dye-binding was seen. Binding to the nonbasic proteins tended to decrease soon after the onset of dye-feeding.

412. STUDIES ON CARCINOGEN-BINDING PROTEINS: I. ISOLATION AND CHARACTERIZATION OF AMINOAZO DYE-BOUND PROTEIN AFTER ADMINISTRATION OF A SINGLE LARGE DOSE OF 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE TO RATS.

Sugimoto T, Terayama H
Fac. Sci. U. Tokyo, Japan
Biochim Biophys Acta; 214(3):533-544 1970

Male rats received 40 mg of 3'-methyl-4-dimethylaminoazobenzene and were killed after 40 hr; liver cell sap was prepared and subjected to heat treatment (55 C for 3 min), followed by chromatography on CM-cellulose. Protein-bound dye was located chiefly in the nonbasic protein fraction, the slightly basic protein fraction, and the more basic protein fractions (Fraction I, IV, VII, and VI, resp). Subsequent purification procedures, including differential precipitation by (NH4)2SO4 or pH adjustment and finally gel filtration were used to separate several dye-binding proteins, I-a, I-b, IV, VII-a and VII-b. The estimated molecular weights

of these dye-binding proteins were 160,000-170,000, 12,000-15,000,160,000-170,000, 160,000-170,000, and 40,000 resp. Proteins I-a, IV and VII-a showed a specific binding of about 1 (mole dye/mole protein) whereas I-b and VII-b showed only a slight modification (0.07 mole dye/mole I-b and 0.01 mole dye/mole VII-b). The isoelectric points of I-a, IV and VII were estimated to be approx. 5.2, 8.0 and 8.6 resp polar dye prepared from I-a, a major dye binding protein under the present experimental conditions, showed mainly P2b and P1 components in addition to P2a, while that prepared from IV or serum gave only P2a and P2a'. Preferential binding to Fraction IV protein was demonstrated for non-carcinogenic 2-methyl-4-dimethylaminoazobenzene.

413. PATTERN OF 2-METHYL-4-DIMETHYLAMINOAZOBENZENE-BINDING PROTEINS IN THE LIVERS OF PARTIALLY HEPATECTOMIZED RATS AND OF CONTINUOUSLY DYE-FED RATS IN COMPARISON WITH 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE.

Sugimoto T, Terayama H
Zool. Inst., Fac. Sci., U. Tokyo, Japan
C R Acad Sci D (Paris); 224(13):1977-1980 1972

Partially hepatectomized and sham-operated rats received 2-methyl-4-dimethylaminoazobenzene (2-Me-DAB) in a single dose (10 mg) and normal rats were fed a diet containing 0.06% 2-Me-DAB for 2-week and 1-month periods. The patterns of dye-binding proteins in the cytosols prepared from the livers of these rats were investigated by chromatography on carboxymethylcellulose as well as by gel filtration in Sephadex G-100, and the results were compared with those of rats given 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) under the same conditions. It was found that 2-Me-DAB given in a single dose is preferentially bound to the slightly basic protein fractions (Fractions IV and VI) in sham-operated rats but to the nonbasic protein fraction (Fraction I) in partially hepatectomized rats, while 3'-Me-DAB is preferentially bound to Fraction I in both cases. Gel filtration of Fraction I revealed that 3'-Me-DAB is preferentially bound to the higher-molecular weight subcomponent (I-a) in both sham-operated and partially hepatectomized rats, while 2-Me-DAB is bound to the smaller-molecular-weight subcomponent (I-b) in the sham-operated rat liver but to I-a in the regenerating liver. For Fraction IV, 3'-Me-DAB is bound to the higher-molecular-weight subcomponent (IV-a) in sham-operated rats but to the lower-molecular-weight subcomponents (IV-b,c) in partially hepatectomized rats, while 2-Me-DAB is bound to the lower molecular-weight subcomponents in both sham-operated and partially hepatectomized rats. The results support a previous finding that dye binding to I-a target protein may be involved in chemical carcinogenesis.

414. MICROELECTROPHORESIS IN AGAROSE GEL OF SOLUBLE RAT LIVER PROTEINS DURING AMINOAZO DYE CARCINOGENESIS.

Louis CJ, Blunck JM
U. Melbourne, Australia
Oncology; 23(4):257-264 1969

A microelectrophoretic method using agarose gel supported on microscope slides is described. In soluble, cytoplasmic proteins of livers from adult, male Sprague-Dawley rats receiving food with 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-MeDAB, up to 12 week), this method, along with autoradiography using tritiated 3'MeDAB, revealed a slight increase in the dye-binding proteins of bands A and B after 2 week, followed by a decrease detectable at 4 weeks. Bands A and B were markedly diminished in hepatoma tissue but normal in surrounding histologically normal liver tissue.

415. TWO SPECIFIC AZODYE-CARCINOGEN-BINDING PROTEINS OF THE RAT LIVER. THE IDENTITY OF AMINO ACID RESIDUES WHICH BIND THE AZODYE.

Ketterer B, Christodoulides L
Middlesex Hosp. Med. Sch. Courtauld Inst. Biochem., London
Chem Biol Interact; 1(2):173-183 1969

Male Wistar rats (200 g) were inj with 3'-methyl-4-dimethylaminoazobenzene (MeDAB; 50 mg in corn oil, ip) and 35S-labeled amino acid (60 microC in saline, ip). The basic and low molecular wt proteins (molecular wt. 45,000 and 13,800, resp) from livers were purified and studied for MeDAB binding. A "fingerprinting" method was used to manipulate the small amounts of bound components after pronase digestion and alkali-ethanol reflux. MeDAB-bound components from both proteins were radioactive after admin of 35S-L-methionine, whereas MeDBA-bound components from the basic protein, only, were radioactive after 35S-L-cysteine admin. It is concluded that MeDAB metabolites bind to cysteine residues in the basic protein, whereas MeDAB binds to methionine residues in the small molecular wt protein.

416. BILIARY EXCRETION OF METABOLITES OF N,N-DIMETHYLAMINOAZOBENZENE (DAB) IN THE RAT (MEETING ABSTRACT).

Levine WG, Finkelstein T
Albert Einstein Coll of Medicine, Bronx, NY 10461
Fed Proc; 36(3):1030 1977

When ^{14}C-DAB is injected iv into rats approximately 25% of the radioactivity appears in the bile within one hour, entirely in the form of metabolites. Among these we have identified azobenzene and the sulfate and glucuronide conjugates of 4'-OH-methylaminoazobenzene. No free or sulfate or glucuronide conjugates of methylaminoazobenzene appear to be excreted, although other unidentified polar metabolites are seen. Biliary excretion of metabolites is more rapid after pretreatment with inducers of drug metabolizing enzymes (phenobarbital, methylcholanthrene) but depressed after enzyme inhibitors (piperonyl butoxide). Bypassing the metabolic steps by injection of preformed metabolites results in a more rapid rate of excretion. Nafenopin treatment, which increases levels of the cytoplasmic azo dye binding protein but not drug metabolizing enzymes does not affect excretion. Based on these findings, it is tentatively concluded that metabolism is the rate-limiting step in the hepato-biliary fate of DAB. Liver GSH has been shown to be increased rapidly after administration of DAB. We have demonstrated that depletion of liver GSH, using iodomethane or diethyl maleate, depresses biliary excretion of DAB metabolites. The mechanism of this effect is under investigation. (Author Abstract)

417. CHEMICAL CARCINOGEN AND ONCOGENIC VIRUS: A POSSIBLE INTERACTION MECHANISM.

Hughes PE
Dept. Path., U. Melbourne, Australia
Nature (Lond); 205:871-874 1965

Swiss albino mice less than 36 hr old were inj ip with a 0.1 ml liver protein preparation from Sprague-Dawley rats: (a) on a control diet; (b) on a control diet + in vitro exposure of the liver proteins to 3'-methyl-4-dimethylaminoazobenzene (3'-MeDAB), (c) on a diet containing 3'-MeDAB (0.06% for 10 days, followed by ip inj of a 2% soln of the carcinogen); or (d) from aminoazo dye -induced hepatomas. The various protein soln mixed with a polyoma virus preparation and allowed to stand for 2 hr at 0 C were then inj into the mice 2 hr after the initial protein soln inj. No tumors occurred in mice given protein with dye adsorbed in vitro, while all the groups of mice given the virus showed typical neck and breast tumors but no

liver tumors. Mice treated with protein from aminoazo dye-fed rat liver protein and viral dilutions of 10-0.75 and 10-0 showed a very high incidence of runting. A 26%-29% tumor incidence occurred with the dye-fed rat protein and viral dilutions of 10-0.75 to -1.5 as compared to a 9%-17% incidence for protein from normal rats; the protein from aminoazo dye-induced hepatomas produced a 27% tumor incidence. The author noted that the experiment was based on the hypothesis that the carcinogen-binding proteins(s) is a repressor of viral oncogenesis.

B. Polycyclic Aromatic Hydrocarbon Binding Sites

418. THE HALF-LIFE OF ARYL HYDROCARBON (BENZO(A)PYRENE) HYDROXYLASE IN HUMAN BLOOD MONOCYTES.

Okuda T, Bast RC, Miller H, Rapp HJ, Gelboin HV
Biology and Chemistry Branches, NCI, NIH, Bethesda, MD 20014
Chem Biol Interact; 14(3-4):379-382 1976

Aryl hydrocarbon (benzo(a)pyrene) hydroxylase (AAH), a mixed-function oxidase system of microsomal cytochrome P-450, is the primary biological receptor for the carcinogenic polycyclic hydrocarbons and may be a key factor in human susceptibility to carcinogenesis. The authors have partially characterized this enzyme in human peripheral blood monocytes. AHH can be induced by treatment with benz-(a)anthracene (BA); therefore, the decay rate of AHH was determined in the presence of both BA and cycloheximide (a potent inhibitor of protein synthesis). AHH was found to have a relatively short half-life. The half-life of AHH from human blood cells is similar to that observed from hamster and rat cells, suggesting that AHH from different species and tissues may have similar stability properties. The relatively short half-life and the highly inducible character of the enzyme support the idea that this system is very sensitive and responsive to xenobiotics in the environment. The degree of responsiveness and enzyme stability may be a factor in human carcinogenesis by environmental chemicals. (15 references)

419. CYTOSOLIC RECEPTOR FOR AH LOCUS: EVIDENCE FOR REGULATORY GENE DYSFUNCTION IN NONRESPONSIVE CELL CULTURES CONTAINING THE RECEPTOR. (MEETING ABSTRACT).

Guenthner TM, Nebert DW
Developmental Pharmacology Branch, NICHD, NIH, Bethesda, MD 20014
Fed Proc; 36(3):648 1977

The ED50 for aryl hydrocarbon hydroxylase (AHH) induction in Hepa-1 and H-4 Reuber cell lines by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is 0.23-0.45 nM and the apparent Kd for TCDD binding to the cytosolic receptor is 0.5-1.0 nM; the ED50 in "nonresponsive" HTC and VERO cell lines is 100- greater than 200 nM and the apparent Kd is 1.5-2.0 nM (Guenthner et al, Fed Proc 35: 282 (1976)). Receptors from responsive and nonresponsive cell lines have similar apparent Kd constant, intracellular concentration, and inducer specificity. Receptors from responsive cell lines differ from those in nonresponsive cell lines with respect to heat lability. During induction of AHH activity in responsive lines by 3-methylcholanthrene or benz(a)anthracene, there is a 50% increase in number of binding sites at 24 and 48 hours; no such increase occurs in the nonresponsive cell lines. Whether this increase in binding sites represents de novo synthesis, activation of preexisting inactive moieties, or decrease in degradation remains to be determined. Certain receptor-containing tissue culture cell line variants therefore possess altered receptor molecules for TCDD, as has been previously shown to be the case for receptors for steroids. These data suggest that mutants in regulatory genes - other than the gene controlling receptor content and/or affinity - are involved in the chronological sequence of events of the induction process in response to polycyclic aromatic hydrocarbons. (Author Abstract)

420. EVIDENCE FOR POSSIBLE RECEPTORS ACTIVE IN ARYL HYDROCARBON HYDROXYLASE INDUCTION IN ESTABLISHED CELL LINES BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (MEETING ABSTRACT).

Guenthner TM, Poland AP, Nebert DW
NICHD, NIH, Bethesda, Md. 20014 and Univ. of Rochester Medical School, Rochester, N.Y. 14642
Fed Proc; 35(3):282 1976

Niwa et al. (Mol. Pharmacol. 11:399-408 (1975)) showed a 10,000-fold difference in the responsiveness of aryl hydrocarbon (benzo(a)pyrene) hydroxylase (AHH) induction to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) among ten established cell lines. Using a radiometric assay for specific binding of (^{3}H)TCDD to cytosol protein, we hoped to determine whether the observed difference in AHH inducibility might be attributed to differences in the number or the affinity of receptor sites. Results indicate the presence of a soluble saturable high affinity binding protein which may act as a receptor in the initial step of AHH induction by TCDD. The apparent Kd for TCDD (0.5-1.0nM) in TCDD-"responsive" Hepa-1 and Reube H-4 lines compared favorably with the ED50 for AHH induction (0.23-0.45 nM). The apparent Kd in TCDD-"nonresponsive" HTC and VERO lines (1.5-2.0 nM), on the other hand, was much less than the ED50 for AHH induction (100-greater than 200 nM), indicating that the inability of these lines to respond to induction by TCDD reflects some defect other than an absence of soluble binding sites. Radiolabelled TCDD was displaced from these sites by nonlabelled TCDD but not by high concentrations of (Author Abstract)

421. SEPARATE IDENTITIES OF LIGANDIN AND THE H-PROTEIN, A MAJOR PROTEIN TO WHICH CARCINOGENIC HYDROCARBONS ARE COVALENTLY BOUND.

Sarrif AM, Danenberg PV, Heidelberger C
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI 53706
Biochem Biophys Res Commun; 70(3):869-877 1976

A protein moiety in the C3H mouse liver cytosol termed mouse ligandin was studied to determine whether it is identical with the h-protein, the major target protein in the mouse liver of methylcholanthrene and its metabolites. Mouse ligandin was identical to a minor methylcholanthrene binding protein species that consists of basic proteins II and III. Both mouse ligandin and mouse h-protein contained glutathione S-transferase activity with different substrate specificities. There was a protein in the mouse liver that was immunologically identical to rat liver ligandin, but it had a separate identity from the h-protein. (31 refs)

422. THE ISOLATION AND CHARACTERIZATION OF POLYCYCLIC HYDROCARBON-BINDING PROTEINS FROM MOUSE LIVER AND SKIN CYTOSOLS.

Sarrif AM, Bertram JS, Kamarck M, Heidelberger C
Med. Sch., Univ. Wisconsin, Madison, WI
Cancer Res; 35(3):816-824 1975

The major protein to which metabolites of methylcholanthrene are covalently bound was purified from C3H mouse liver cytosol. The purification procedure involved successive fractionations on Sephadex G-25, DEAE-cellulose, and Sephadex G-100. Following the last step, the protein was subjected to carboxymethyl-cellulose chromatography, a second Sephadex G-100 fractionation, and sodium

dodecylsulfate-polyacrylamide gel electrophoresis. The properties of the protein were identical to those of mouse skin h-protein, which may be a primary target of carcinogenic hydrocarbon metabolites during transformation to cancer. It had a molecular weight of 44,000, consisted of two subunits of molecular weight 20,000 each, had an isoelectric point (pI) of 8.5-8.6, and a sedimentation coefficient of 3.6 S. These properties are rather similar to those of ligandin, a rat hepatic protein that binds carcinogenic metabolites, steroid anionic metabolites, bilirubin, and exogenous organic anions. However, the h-protein and ligandin consistently gave different pI values (average of 8.3 and 8.9, resp). The mouse h-protein was not similar in physical properties to the rat liver azo dye carcinogen binding "slow H2-5S" protein. Two minor basic proteins (molecular weight around 44,000 each), to which methylcholanthrene metabolites are covalently bound, were separated from the h-protein by carboxymethyl-cellulose chromatography. Preliminary results indicated that these two minor proteins are identical to ligandin. A protein to which methylcholanthrene is noncovalently bound was also identified in the acidic fraction of the mouse liver and skin cytosols. It had a molecular weight of 60,000, a pI of 5.0, and a sedimentation coefficient of 4.5 S. The protein may be similar to a previously- described protein to which carcinogenic hydrocarbons bind specifically in vitro. Whether any of these proteins play a role in carcinogenesis is yet to be determined.

423. THE POSSIBLE ROLE OF HYDROPHOBIC INTERACTIONS OF POLYCYCLIC AROMATIC HYDROCARBONS WITH PROTEIN IN CHEMICAL CARCINOGENESIS.

Franke R
Tech. Univ., Dresden, Germany
Mol Pharmacol; 5(6):640-657 1969

The interrelationship of 34 polycyclic aromatic hydrocarbons and their carcinogenic activity, chemical reactivity and hydrophobic interactions with protein were investigated using methods of partial and multiple correlation and regression analysis. Hydrophobic interaction was found to be necessary for chemical carcinogenesis, possibly due to the induction of a conformational change in a receptor protein, but was of secondary importance compared to the chemical reactivity of the hydrocarbons. An index was presented which grouped hydrocarbons according to carcinogenic activity and described variations in activity due to polar or alkyl group substituents. The results were consistent with the K-L-region theory and the protein deletion hypothesis.

424. INHIBITION BY DEXAMETHASONE OF INTRACELLULAR BINDING OF PHORBOL ESTERS TO MOUSE SKIN.

Slaga TJ, Scribner JD, Rice JM, Das SB, Thompson S
Fred Hutchinson Cancer Res. Ctr., Seattle, Wash.
J Natl Cancer Inst; 52(5):1611-1618 1974

The binding of phorbol esters to Charles River mice epidermal subcellular fractions and the effect of tumor promoters on the protein complement of cytosol were investigated. When applied to mouse skin, 14C-labeled 12-O-tetradecanoylphorbol-13 acetate (TPA, 17 Nmoles; 2.6 X 105 cpm) and 3H-labeled 12-O-hexadecanoylphorbol-13 acetate (HPA, 16Nnmoles; 2.3 X 106 cpm) remained associated, after extensive dialysis, with epidermal chromatin, cytosol, microsomes, and mitochondria. The specific activity of TPA and HPA binding to the subcellular fractions reached a peak by 1 hr after treatment. The powerful tumor promoter TPA had a greater affinity for the subcellular fractions than did the weaker promoter HPA. Simultaneous application of the antipromoting agent dexamethasone (225 Nmoles) inhibited the binding to the cytosol and chromatin fractions from 1 to 24 hr after treatment but did not affect the other fractions. Label continued to be associated with cytosol protein after removal of unbound radioactivity by dialysis, gel filtration, and ultrafiltration. Polyacrylamide gel electrophoresis of the promoter-bound cytosol protein showed that most radioactivity was associated with the protein fraction previously reported to be a receptor for 3-methylcholanthrene. TPA and HPA binding to the cytosol receptor protein was inhibited between 3 and 12 hr after dexamethasone treatment. In vitro competition by a promoter or a nonpromoter for binding of radioactive TPA to the cytosol receptor protein correlated with promoting activity. However, dexamethasone did not compete in vitro for TPA binding to the cytosol receptor protein. When the protein complement of mouse epidermal cytosol was analyzed by electrophoresis on two different polyacrylamide gels, dexamethasone inhibited a slowly migrating fraction which was most enhanced between 12 and 24 hours after treatment. The findings suggest that both the chromatin and cytosol fractions are involved in the early action of skin tumor promoters.

425. PHYSICAL BINDING OF LABELED 20-METHYLCHOLANTHRENE TO MOUSE EPIDERMAL SUBCELLULAR FRACTIONS.

Slaga TJ, Scribner JD, Rice JM
Pacific Northwest Res. Fdn., Seattle, Wash.
Cancer Res; 33(5):1032-1037 1973

The level and degree of selectivity of noncovalent binding that might take place between a carcinogenic hydrocarbon and mouse epidermal subcellular fractions was examined. When applied to the skin of female Charles River CD1 mice, 3H-labeled 20-methylcholanthrene (MC) is physically absorbed by the chromatin, mitochondria, microsomes, and cytosol of mouse epidermis. Label remained associated with cytosol protein after removal of free MC and any low-molecular-weight metabolites by dialysis, gel filtration, and ultrafiltration; this association reached a maximum around 3 hr after application of MC to mouse skin. Although previous work reported covalent binding to mouse skin cytosol, most of the material bound to cytosol protein after mild isolation of cytosol and removal of low-molecular-weight radioactivity is easily removed by precipitation of the protein with ethanol or trichloroacetic acid. Enzymatic digestion established that most of the MC loosely bound to the cytosol or chromatin was in fact bound to protein. Electrophoresis of the MC-bound cytosol protein on 7% basic polyacrylamide gels showed that most of the activity was associated with a protein fraction that had the same mobility as bovine or mouse serum albumin. The binding to the cytosol fraction and to chromatin was inhibited by the simultaneous application of 7,12-dimethylbenz(a)anthracene or the promoter 12-O-tetradecanoyl-phorbol-13-acetate, and was less inhibited by benz(a)anthracene or the nonpromoter phorbol-13,20-diacetate. The binding to the cytosol receptor protein and to chromatin may be involved in the promoting stage of mouse skin tumorigenesis.

426. IN VITRO BINDING OF LABELED 20-METHYLCHOLANTHRENE TO A SPECIFIC CYTOSOL-PROTEIN.

Slaga TJ, Scribner JD, Rice JM
Pacific Northwest Res. Fdn., Seattle, Wash.
Chem Biol Interact; 7(1):51-62 1973

The in vitro binding kinetics and specificity of interaction of the 3H-labeled carcinogen, 20-methylcholanthrene (MC), with mouse epidermal cytosol in the presence or absence of a purified mouse microsomal metabolizing system were studied. Following incubation the samples were dialyzed, subjected to gel filtration and ultrafiltration, and analyzed by basic polyacrylamide gel electrophoresis. A major part of the

binding appeared in a single peak on the gel which had the same mobility as bovine and mouse serum albumin. The in vitro binding of MC to the cytosol protein could be inhibited by other hydrocarbons or promoters to degrees which correlated with their carcinogenicity or promoting ability. Essentially all of the MC which bound to cytosol in the absence of a microsomal metabolizing system was non-covalently bound. However, binding of MC to cytosol in the presence of microsomes plus NADPH was partly covalent as previously reported by others. When bovine or mouse serum albumin was incubated with 3H-MC in the presence of competing non-radioactive carcinogens or promoters, little or no inhibition of binding was observed. It was also found in a preliminary experiment that radioactive 12-O-tetradecanoylphorbol-13-acetate (TPA), a promoter, was also bound primarily to the cytosol receptor protein when applied to mice or incubated with epidermal cytosol. It is concluded that the specific binding of MA and TPA to the mouse epidermal cytosol receptor protein may represent a critical interaction for the promotion stage of chemical carcinogenesis in mouse skin.

427. IDENTITY OF CORTICOSTEROID BINDER I WITH THE MACROMOLECULE BINDING 3-METHYLCHOLANTHRENE IN LIVER CYTOSOL IN VIVO.

Singer S, Litwack G
Temple U. Sch. Med., Philadelphia, Pa.
Cancer Res; 31(10):1364-1368 1971

"Corticosteroid Binder I" has been shown to be identical to the protein which is able to bind the carcinogen, 3-methylcholanthrene (MC), in rat liver. When binder I was purified from hepatocytes of rats simultaneously labeled with ^{14}C-MC and 3H- cortisol, both labels were recovered. Cortisol- and MC-binding proteins, purified on Sephadex G-75 columns, showed the same migration pattern when subjected to isoelectrofocusing. Purified MC-binding protein also bound labeled corticosterone. The molecular weights of the two protein-bound complexes were the same, approx 38,000, and agreed with values previously reported.

428. ALTERED HORMONE CONTROL OF CYCLIC AMP FORMATION IN ISOLATED PARENCHYMAL LIVER CELLS FROM RATS TREATED WITH 2-ACETYLAMINOFLUORENE.

Christoffersen T, Berg T
Inst. Pharmacology, Univ. Oslo, Norway
Biochim Biophys Acta ; 381(1):72-77 1975

Parenchymal liver cells isolated from adult male Wistar rats fed a special diet supplemented with 2-acetylaminofluorene (0.025% by weight) for 4-6 wk were studied to determine the responsiveness of adenylate cyclase to adrenergic versus glucagon stimulation. Liver cells were dispersed by perfusion in vitro with 0.05% collagenase and purified by filtration and centrifugation. After preincubation for 45 min, 3.75 x 10^6 cells were incubated with stimulators and/or inhibitors in 2 ml buffer containing 2 mM theophylline. Cyclic AMP was determined by a protein-binding assay validated for crude extracts of liver cells. Glucagon (1.4 x 10**-6 M) gave 20 pM cyclic AMP/10^6 cells versus a basal level of 2 pM cyclic AMP/10^6 cells in cells from the untreated group, but 5 x 10**-5 M adrenalin or isoprenalin resulted in levels of cyclic AMP of 2 and 3 pM, respectively. Cells from the treated group showed a marked increase in cyclic AMP levels in response to adrenalin (15 pM) and isoprenalin (17 pM). Cyclic AMP levels in cells from treated rats were 16, 14, and 3 pM/10^6 cells after addition of 10**-6 M adrenalin, isoprenalin, or phenylephrine, respectively. Cyclic AMP was 17 pM/10^6 cells with 1.4 x 10**-6 M glucagon. The effects of phenylephrine and isoprenalin were blocked by D, L-propanolol but not by phentolamine, indicating that the receptor has predominantly b-characteristics. When 1.4 x 10**-6 M glucagon was added with 10**-5 M isoprenalin, no additive effect was noted. This lack of additive effect indicates that the hormones act on one cell population. While parenchymal cells from normal rat liver respond strongly to glucagon and poorly to adrenalin, those of treated rats have become responsive to adrenergic agents. (20 refs)

VI. PROTEINS WHICH BIND VITAMINS AND/OR COFACTORS

A. Retinoic Acid Binding Proteins

429. RETINOIC ACID BINDING PROTEIN: OCCURRENCE IN HUMAN TUMORS.

Ong DE, Page DL, Chytil F
Vanderbilt Univ. Medical Sch., Nashville, TN 37232
Lab Invest; 34(3):329 1976

Soluble tissue extracts (in 0.05 M Tris-HCl, pH 7.5) were tested for the presence of a binding protein for retinoic acid (vitamin A acid) by incubating aliquots with 40 nmoles of all-trans(11,12-^{3}H)retinoic acid in the dark. In order to determine specific binding, duplicate aliquots containing 8 umoles of unlabeled retinoic acid were used. Free retinoic acid was absorbed by charcoal dextran solution after incubation. An aliquot of supernatant was layered on a linear 5 to 20% sucrose gradient of 5 ml and centrifuged at 148,000 x g for 20 hr. The presence of the binding protein is indicated by a peak of radioactivity in the 2 S region of the gradient which is reduced by the addition of unlabeled retinoic acid. A second peak of 4.6 S is frequently observed and, in contrast to the 2 S peak, this 4.6 S binding is not diminished by excess unlabeled retinoic acid. Malignant tissue demonstrated a peak of radioactivity sedimenting in the 2 S region of the gradient. Normal tissue from the same organ, monitored histologically for epithelial content, lacked the specific binding protein at 2 S. A total of four carcinomas have been examined (one lung, three breast) and all showed 2 S binding indicative of the retinoic acid binding protein. We conclude that at least in these malignant tumors there is an altered interaction of retinoic acid within the cell compared to normal tissue.

430. RETINOIC ACID BINDING PROTEIN (RABP) IN NORMAL, PRENEOPLASTIC AND NEOPLASTIC HUMAN MAMMARY TISSUE (MEETING ABSTRACT).

Huber PR, Geyer E, Matter A, Eppenberger U
Hormonlabor und Experimentelle Endokrinologie,
Universitats-Frauenklinik, CH-4004 Basel, Switzerland
Experientia; 33(6):821 1977

Tumor growth is recognized to occur in three distinct phases: initiation, preneoplasia and transformation. Recently Vitamin A and its oxidation product retinoic acid have been implicated to be involved in the initiation of tumors; ie retinoic acid could inhibit and reverse tumor growth. However, the molecular mechanisms by which retinoids act remain to be established. The detection of a specific retinoic acid binding protein (RABP) in human breast and lung tumor, but not in the surrounding tissue may indicate that retinoids exert their physiological effect by such binding proteins. Though the molecular mechanism is still unknown we have strong evidence that the appearance of RABP parallels the developing of tumors. In normal tissue of eight patients no RABP could be detected, but 55% of proliferative dysplasias (20 patients) and 53% of primary carcinomas (21 patients) contained RABP. The binding protein was determined with agar gel electrophoresis and sucrose density gradients. (no Refs)

431. MEDIATION OF RETINOIC ACID-INDUCED GROWTH AND ANTI-TUMOUR ACTIVITY.

Chytil F, Ong DE

Dept. Biochemistry, Vanderbilt Univ. Nashville, TN 37232

Nature (Lond); 260(5546):49-51 1976

The mechanism of action of retinoic acid was studied. Newborn mouse skin, hamster trachea, and papillomas of mouse skin were examined for the presence of cellular retinoic acid-binding protein. With the use of extracts of mouse skin papillomas, human breast tumor, and a thousandfold purified preparation from rat testis as sources for the binding protein, its relative affinity for ring analogs of retinoic acid was determined. The binding characteristics of the protein toward the analogs correlated with the activity of the compounds. Analysis of extracts of newborn mouse skin and of hamster trachea revealed a peak of radioactive retinoic acid in the 2 S region of the sucrose gradient, indicating the presence of cellular retinoic acid-binding protein. Mouse skin papillomas had a high level of the binding protein compared with the control skin of mice. Both the dimethylacetylcyclopentenyl and trimethylmethoxphenyl analogs of retinoic acid were effective competitors for the binding of retinoic acid. The phenyl, furyl, and pyridyl analogs were less effective. The presence of the binding protein suggests that it may mediate the action of retinoic acid. (14 Refs)

432. TISSUE DISTRIBUTION AND POSSIBLE SIGNIFICANCE OF RETINOIC ACID-BINDING PROTEIN.

Sani BP

Kettering-Meyer Lab., Southern Research Institute, Birmingham, AL 35205

Proc Am Assoc Cancer Res; 17:8 1976

Evidence presented from this laboratory (BBRC, 61, 1276, 1974) concerned the detection and partial characterization of a retinoic acid-binding protein (RABP) from chick embryo skin. The ability of various analogs of retinoic acid (RA) to bind to this protein correlated with their biological activity in inhibition of keratinization of chick embryo skin in organ culture (Wilkoff et al, Cancer Res, in press). Studies on tissue distribution of RABP, which has an S20 value of 2, in tissues of young and adult rats and mice and chick embryos revealed its presence in the skin, brains and testes of these species. A relatively large amount of RABP was present in these tissues of young animals, a fact consistent with the involvement of RABP-RA complex in growth and/or differentiation. In the skin, RABP was located in the epithelial cells and not in the connective tissue, in agreement with the observation that RA is involved in epithelial differentiation. RABP was present in small quantities in the trachea, bladder, prostate, uterus and mammary gland of rats. In all other tissues tested, including lung, liver, heart, intestine and kidney, it could not be detected. However, Lewis lung tumor and lungs with metastatic foci contained RABP. (Author Abstract)

433. EFFECT OF COLCHICINE (COL) ON RETINOL-BINDING PROTEIN (RBP) METABOLISM (MEETING ABSTRACT).

Smith JE, Navab M, Goodman DS

Columbia Univ College of P and S, NY, NY 10032

Fed Proc; 36(3):1102 1977

Vitamin A deficient rats have low serum RBP levels and an expanded pool of liver RBP. Injection of retinol in 20% Tween-40 into vit A deficient rats resulted in the rapid mobilization of RBP from liver into serum. The effect of COL, an agent known to disrupt microtubules, on the retinol-stimulated release of RBP was studied. Vit A deficient rats were injected ip with isotonic NaCl or with COL (5 mg/kg body wt). 3 hrs later the rats were injected iv with Tween alone or with 50 mug of retinol in Tween, and blood and liver samples were collected 90 min later. The effect of COL on RBP secretion was compared to its effects on triglyceride (reflecting very low density lipoprotein) secretion and on total protein synthesis by the liver. Injection of retinol caused serum RBP levels to increase from 6 plus or minus 1 mug/ml to 53 plus or minus 5 (mean plus or minus SEM) within 90 min. The increase in serum RBP was much smaller (only 36% as great, to 23 plus or minus 3 mug/ml) in rats given COL prior to retinol injection. A quantitatively similar inhibition of the release of triglyceride by liver into serum was observed in COL-treated rats, as also reported by Stein et al (J Cell Biol 62:90, 1974). In contrast, COL did not inhibit the incorporation of ^{3}H-leucine into total liver plus serum proteins, but it did inhibit hepatic release of newly synthesized proteins. These data support the hypothesis that microtubules are involved in the secretion of RBP from the liver. (Author Abstract)

434. QUANTITATION OF BINDING AFFINITIES OF RETINOIDS TO RETINOIC ACID-BINDING PROTEIN AND ALBUMIN (MEETING ABSTRACT).

Sani BP, Titus BC

Southern Res Inst, Birmingham, AL 35205.

Proc Am Assoc Cancer Res; 18:42 1977

The binding of retinoic acid (RA) and its synthetic analogs to intracellular RA-binding protein (RABP) and to serum albumin has been assessed. RA is known to be involved in epithelial differentiation and tumorigenesis. A quantitative method, involving immunoprecipitation of serum albumin, now developed for measuring the binding efficiency of various retinoids to RABP may be useful in assessing their biological potencies. The binding affinities of some retinoids are as follows: Cyclopentenyl analog of retinoic acid greater than all-trans-RA = 13-cis-RA greater than trimethyl methoxyphenyl (TMMP) analog of RA greater than dichlorophenyl analog of RA greater than TMMP analog with a sulphur atom in the side chain greater than phenyl analog or RA. Except for thioctic acid, none of several vitamins, hormones, and cofactors showed appreciable affinity for the RA-binding site on RABP. Since the binding affinity of RABP for RA analogs correlates with their biological activity, it was desirable to determine their binding to serum albumin, which facilitates the transport of these retinoids. RA and its phenyl analog showed a similar degree of competition for albumin binding. The TMMP and dichlorophenyl analog of RA and 13-cis-RA competed even better than RA for the binding sites. Since some of these analogs have only very little biological activity, such activity is not likely to be directly associated with binding to serum albumin. (Author Abstract)

435. EFFECTS OF LIVER STORES, DIETARY INTAKE, AND TISSUE UTILIZATION OF VITAMIN A IN THE REGULATION OF PLASMA VITAMIN A LEVELS IN RATS (MEETING ABSTRACT).

Keilson B, Lewis KC, Underwood BA, Loerch JD

The Pennsylvania State University, University Park, PA 16802

Fed Proc; 36(3):1135 1977

Male weanling Sprague-Dawley and Sherman rats were fed diets sufficient (3 mug/g) or deficient (0 mug/g) in vitamin A for 3 1/2-6 weeks. Sub-groups of these rats received a dietary supplement of retinoic acid (12 mug/g) for the entire experiment or after the 3rd week. Plasma and liver vitamin A levels were determined periodically. Plasma levels held steady (about 45 mug/100 ml) in rats fed the deficient diet until liver stores dropped below 60 mug/liver (6 mug/g liver). Dietary retinoic acid lowered plasma levels of vitamin A at least 10 mug/100 ml in all rats and had a sparing action on liver stores. The short-term plasma dose response to a vitamin A challenge was measured in sub-groups of these rats over a 5-hour period.

Both ^{3}H-retinyl ester (4.8 mug in chylomicrons, injected intrajugularly) or unlabeled retinyl acetate (20 mug in oil, given by stomach tube) produced a surge in circulating retinol levels. Both the magnitude and duration of the response were higher in rats having low liver stores regardless of retinoic acid supplementation. A model is proposed in which plasma retinol levels are regulated in part by the rate of retinol utilization in tissues, whereas short-term mobilization of newly-ingested vitamin A is determined primarily by levels of hepatic stores, possibly through the mediation of available retinol-binding protein as suggested by others. (Author Abstract)

436. PROPERTIES OF CELLULAR RETINOIC ACID BINDING PROTEIN FROM RAT TESTIS AND EPIDIDYMIS (MEETING ABSTRACT).

Ong DE, Chytil F

Dept of Biochemistry and Med, Vanderbilt Univ School of Med, Nashville, TN 37232

Fed Proc; 36(3):1136 1977

Previous work from this laboratory has shown the existence of the two highly specific cellular binding proteins, one for retinol (CRBP) and one for retinoic acid (CRABP), in various animal organs including mature rat testes. These proteins are distinct from the serum retinol binding protein. The binding proteins are detected by sucrose gradient centrifugation of organ extracts preincubated with tritiated retinol or retinoic acid. Both proteins have a sedimentation constant of about 2 S. Extracts of adult rat epididymis also contain a binding protein for retinol and one for retinoic acid, both of which sediment in the 2 S region of sucrose gradients. The elution position of CRBP (testis) and CRBP (epididymis) on a Sephadex G-75 column is consistent with a molecular weight of 14,000. The elution position of CRABP (testis) is equivalent to a molecular weight of about 15,000 but CRABP (epididymis) elutes earlier, equivalent to a molecular weight of about 20,000. The data show that testicular CRABP is not identical with epididymal CRABP, suggesting that this protein might have different properties depending on the organ. (Author Abstract)

437. PRESENCE OF CELLULAR RETINOL AND RETINOIC ACID BINDING PROTEINS IN EXPERIMENTAL TUMORS.

Ong DE, Chytil F

Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN 37232

Cancer Lett (Amsterdam); 2(1):25-30 1976

The role of vitamin A in the development of epithelial tumors may be related to cellular binding proteins. Experimental tumors were studied to determine the presence of two binding proteins, one that specifically binds retinol and another that specifically binds retinoic acid. Mouse skin papillomas were induced on the backs of Swiss albino mice. The following tumors were grown as transplants: Ehrlich carcinoma and sarcoma 180 in Swiss albino mice; leukemia L1210 in BDF mice; human adenocarcinoma HAD-1 in hamsters; Walker 256 carcinoma in Sprague-Dawley rats; chondrosarcoma, mammary adenocarcinoma MAC-1, and Dunning leukemia in F-344 rats. Tumor tissue was excised immediately after the hosts were killed and kept at -20 C until assayed. Tumor tissue was homogenized with 0.05 M Tris-HCl. Samples of the extracts were incubated with 60 pmol all-trans-(15-^{3} H)retinol or all-trans(11,12-^{3} H)retinoic acid. Parallel incubations contained a hundredfold excess of unlabeled retinol or retinoic acid. Aliquots were layered on a sucrose gradient and centrifuged for 20 hr. The gradients were fractionated and the radioactivity was determined by liquid scintillation counting. The binding proteins were detected as a peak of radioactivity in the 2S region of the gradient. Cellular retinol and retinoic acid binding proteins were detected in mouse skin papillomas, human adenocarcinoma HAD-1, Dunning leukemia, Walker 256 carcinosarcoma, and mammary adenocarcinoma MAC-1. A chondrosarcoma and sarcoma 180 apparently contain only the cellular retinoic acid binding protein. Neither protein was detected in Ehrlich carcinoma and L1210 leukemia. It is proposed that the presence of the cellular retinoic acid binding protein may be necessary for a tumor to be sensitive to treatment with retinoic acid or its analogs. The presence of both binding proteins in some tumors suggests that simultaneous administration of retinol and retinoic acid derivatives may provide a better prophylactic or therapeutic effect. (42 refs)

438. RETINOIC ACID-BINDING PROTEIN IN NORMAL TISSUES AND EXPERIMENTAL TUMORS.

Sani BP, Corbett TH

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Cancer Res; 37(1):209-213 1977

The tissue distribution of retinoic acid-binding protein (RABP) has been determined for tissues of chick embryos and young and adult rats and mice and has been compared with other published data. Although no species variability has been detected with tissue distribution of RABP, relatively more of the protein is detected in the tissues of young animals than in those of adult ones. This protein is below the limits of detection in the adult rat or mouse brains, whereas it was present in abundance in the embryonic and young brains. RABP is present in the epithelial cells but not in the connective tissue of skin. Besides brain, skin, testis, and eye, RABP is also detected in small quantities in the bladder, prostate, uterus, trachea, and mammary glands of rats and mice. Although RABP could not be detected in normal lungs, this protein is found to be present in Lewis lung tumors and in lungs with metastatic Lewis lung foci. Of four chemically induced transplantable colon tumors of mice, two highly metastatic ones contained RABP, whereas the two nonmetastatic lines as well as normal colon did not. (Author Abstract)

B. Vitamin B12 Binding Proteins

439. ATYPICAL PLASMA FACTOR ASSOCIATED WITH BRONCHOGENIC CARCINOMA AND COMPLEXING WITH R-TYPE VITAMIN B12-BINDING PROTEINS.

Jacob E, Herbert V, Burger RL, Allen RH

Veterans Admin. Hosp., 130 W. Kingsbridge Road, Bronx, NY 10468

N Engl J Med ; 296(16):915-917 1977

The plasma of a 59-yr-old man with bronchogenic carcinoma was investigated. His plasma was abnormal in that no vitamin B12 or vitamin B12 binding ability eluted in the 150,000 dalton molecular wt R type protein region and in that increased amounts of vitamin B12 and vitamin B12 binding protein ability eluted in the void of the gel filtration column. The vitamin B12 that eluted in the void had the antigenic properties of R type protein; this suggested that the plasma might contain a factor that bound to R type protein and shifted its elution position to the void region. When the plasma was adjusted to contain 40% ammonium sulfate followed by centrifugation, more than 90% of the R type protein binding factor was recovered in the pellet. An extract of 200 mg of tumor contained 2,000 picog of B12 binding ability. A value of 400 picog was obtained with similar treatment of a lung tumor. (7 refs)

440. HUMAN PLASMA R-TYPE VITAMIN B12-BINDING PROTEINS.

Burger RL, Schneider RJ, Mehlman CS, Allen RH
Divisions of Hematology and Oncology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110
J Biol Chem; 250(19):7707-7713 1975

The normal human granulocyte vitamin B12-binding protein transcobalamin I, and transcobalamin III, have been labeled with ^{125}I-labeled N-succinimidyl 3-(4-hydroxyphenyl)propionate and utilized for plasma clearance studies performed with rabbits. Both moieties of ^{125}I-labeled granulocyte vitamin B12-binding protein (^{57}Co)vitamin B12 were cleared rapidly from the plasma (greater than 90% by 5 min) by the liver. After 30 min, the bulk of the ^{125}I reappeared in the plasma in small molecular weight (less than 1000) form and was rapidly excreted in the urine. After 60 min the bulk of the (^{57}Co)vitamin B12 reappeared in the plasma bound to rabbit transcobalamin II and was subsequently taken up by a variety of tissues. Approximately 15% of the ^{125}I-labeled granulocyte vitamin B12-binding protein (^{57}Co)vitamin B12 was excreted intact into the bile during the period from 10 to 80 min after injection. The hepatic uptake of the protein vitamin B12 complex was blocked by the prior injection of desialyzed fetuin but not by native fetuin. Similar results were obtained with ^{125}I-labeled transcobalamin III (^{57}Co)vitamin B12. Approximately 90% of both moieties of ^{125}I-labeled transcobalamin I (^{57}Co)vitamin B12 had prolonged plasma survivals similar to that of ^{131}I-labeled bovine serum albumin. After treatment with neuraminidase, both moieties of the ^{125}I-labeled transcobalamin I (^{57}Co)vitamin B12 complex were cleared rapidly from the plasma by the liver in a manner that was indistinguishable from that observed in the case of untreated granulocyte vitamin B12-binding protein and transcobalamin III. These observations indicate that desialyzed transcobalamin I and the native forms of the granulocyte vitamin B12-binding protein and transcobalamin III are cleared from plasma by the mechanism elucidated by Ashwell and Morell (Ashwell G and Morell AG, Adv Enzymol; 41:99-128 1974) that is capable of clearing a wide variety of asialoglycoproteins. These observations have implications concerning the function of the human R-type vitamin B12-binding proteins, the nature of the enterohepatic circulation of vitamin B12, the biologic significance of the mechanism described by Ashwell and Morell, and the etiology of the increased plasma concentration of human R-type protein that occurs frequently in chronic myelogenous leukemia and occasionally in hepatocellular carcinoma and other solid tumors. (Author Abstract)

441. STUDIES ON THE ABNORMAL HIGH BINDING CAPACITY OF BLOOD FOR VITAMIN B12 IN CHRONIC MYELOID LEUKEMIA.

Fischer E
Neurochem. Inst., Copenhagen, Denmark
Clin Chim Acta; 36(2):409-418 1972

Experiments were designed to examine the binding of vitamin B12 to the alpha-globulin blood serum fraction in patients with chronic myeloid leukemia. Serum from 47 chronic myeloid leukemia patients was used. Assays of B12 in these sera showed that serum B12 values were increased in the leukemic patients; 91% of patients showed a serum vitamin B12 level above 2000 pg/ml, with B12 levels in normal serum samples averaging 455 pg/ml. No significant correlation was found between numbers of leucocytes in patients' samples and B12 serum values. The B12 binding capacity of serum was further investigated on leucocytes isolated from normal individuals and from chronic myeloid leukemia patients. The relative mobilities and relative percentage distribution of protein fractions separated by means of agar gel microelectrophoresis of concentrated leucocyte extracts from normals and chronic myeloid leukemia patients were examined. The leucocytes from leukemia patients revealed several more protein fractions in the electrophoretic mobility interval from 0.06-0.89 than occurred in normal leucocytes. This was the only distinct B12 binding protein with an alpha-globulin mobility shown by either normal leucocytes or patients' leucocytes. In immunoelectrophoretic analysis of leucocyte extracts (from normals and patients) incubated with 58Co-labelled B12, only one B12 binding protein was demonstrated. The finding that there is only one vitamin B12 binding protein with a mobility in normals as well as in chronic myeloid leukemia patients suggests that in chronic myeloid leukemia an increased number of leukemic leucocytes will liberate vitamin B12 binding protein to the blood stream rather than liberating an abnormal leukemic protein with high serum B12 binding capacity.

442. SERUM VITAMIN B12-BINDING PROTEINS IN A CASE OF EOSINOPHILIC LEUKEMIA.

Coltman CA, Panettiere F, Carmel R
Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, Texas 78236
Med Pediatr Oncol; 1(3):185-193 1975

A 24-yr-old male patient is described with subacute eosinophilic leukemia. Serum vitamin B12 and B12 -binding protein studies and simultaneous complete blood counts were done before and during 6 months of high-dose, intermittent combination chemotherapy. The patient presented with extremely high levels of serum vitamin B12 , unsaturated B12 -binding capacity, and transcobalamin I, all of which resembled the highest values seen in chronic myelogenous leukemia. Serial studies, during and after remission induction, showed a precipitous fall of serum vitamin B12 and unsaturated B12 -binding capacity to normal levels. Transcobalamin I levels, which eventually reached low-normal range, correlated best with the level of circulating and bone marrow eosinophils. Transcobalamin II and serum third binder appeared to be normal throughout the patient's course. The B12 -binding protein abnormalities are not considered diagnostic of eosinophilic leukemia.

443. COMPARISON OF BIOCHEMICAL PROPERTIES OF HUMAN SERUM B12 BINDERS DERIVED FROM SEPHADEX AND DEAE-CELLULOSE CHROMATOGRAPHY.

Kumar S, Meyer LM, Gams RA
Perinatal Lab., Christ Hosp., 4440 W. 95th St., Oak Lawn, IL 60453
Scand J Haematol; 18(5):403-412 1977

The biochemical properties of human serum B12 binders (TC) derived from Sephadex and diethyl amino ethyl (DEAE)-cellulose chromatography were studied. Gel-filtration on Sephadex G200 separated the binding proteins into two peaks, one corresponding to small (TCS) and large (TCL) molecular wt fractions. Upon chromatography on DEAE-cellulose, three peaks were recovered from both TCS and TCL. TCS peaks had an apparent molecular wt of approx 40,000, while those from TCL had a molecular wt of 110,000. On paper electrophoresis, peaks eluted with 0.06 M phosphate buffer, (pH 5.85) from both TCS and TCL moved as beta-globulins; those eluted with 0.1 M buffers (pH 5.8) between beta- and alpha2-globulin, and those eluted with 0.25 M buffer (pH 5.4) between alpha1-and alpha2-globulin. TCS was active in delivering Co57-B12 to L-1210 leukemic lymphoblasts while TCL showed no such activity. Labeled B12 was delivered to these cells only by the peaks eluted with 0.06 M and 0.1 M buffers. Only the peaks eluted with 0.06 M and 0.01 M buffers, TCS and TC II B12 reacted with anti-TC II B12. (26 Refs)

444. THE CLINICAL AND PHYSIOLOGICAL IMPLICATIONS OF HEPATOMA B12-BINDING PROTEINS.

Waxman S, Liu C, Schreiber C, Helson L
Cancer Chemotherapy Foundation Lab, Div of Medical Oncology, Dept of Medicine, Mount Sinai Sch of Medicine, City Univ of New York, NY 10029
Cancer Res; 37(6):1908-1914 1977

A serum B12-binding protein with increased sialic acid content (termed hepatoma B12-binding protein) that causes elevations of serum B12 and unsaturated B12-binding capacity has been found in some patients with hepatocellular carcinoma (hepatoma). We now report another patient with hepatoma with initial near-normal, unsaturated B12-binding capacity that increased 400-fold as the disease progressed and then fell 50% with response to chemotherapy. A perfusate of the tumor in the liver had 5 times more B12-binding protein than did the serum and was immunologically the same as the serum hepatoma B12-binding protein isolated from previous cases. A cell line derived from hepatoma produced significant amounts of B12-binding protein similar to hepatoma B12-binding protein, whereas cell lines from normal liver and other neoplasia did not. The hepatoma sera, perfusate, and media from the hepatoma cell line contained elevated sialyltransferase activity. These data suggest that some hepatomas produce increased hypersialylated B12-binding protein that is cleared slowly from the plasma and accumulates there as hepatoma B12-binding protein. (17 Refs) (Author Abstract)

445. NOVEL GLYCOPROTEINS IN HEPATOCELLULAR CARCINOMA (HEPATOMA) (MEETING ABSTRACT).

Liu CK, Schreiber C, Schmied R, Waxman S
Mount Sinai Sch of Medicine, New York, N.Y. 10029.
Proc Am Assoc Cancer Res; 18:231 1977

Elevated levels of hypersialylated B12 binding protein (B12BP) and sialyltransferase activity (SA) are present in some patients with hepatoma (J. Clin. Invest. 56:1262, 1975). A cell line derived from a patient with hepatoma secretes specific hepatoma B12BP and SA into its culture medium. B12BP or SA are not secreted in significant amounts by cell lines derived from neuroblastoma, lymphocytes, leukemia, NRK, or XC. B12BP and SA appear in the hepatoma culture medium after 24 hours growth, increase 5-fold, and are proportionate to the cell growth. The addition of cAMP or cGMP (5 x10**-5M) results in a 2-fold increase in B12BP with only slight increase in SA. The media contains the small form of SA (70,000 MW) in contrast to cellular SA which has a large (greater than 200,000 MW) species in addition. This SA secreted in the media binds to Concanavalin-A Sepharose and elutes in two fractions (0.2M NaCl or 5% alpha-methylmannoside with 0.2M NaCl). The eluted SA gives different patterns with at least two bands of SA on polyacrylamide gel. SA is thought to be glycoprotein, and the multiple species of SA may result from differences in their carbohydrate contents. The data suggest that B12BP and SA may be sensitive markers for certain hepatomas. The significance of the multiple species of SA and their role in the tumorigenesis of hepatoma needs further study. (Author Abstract)

446. HUMAN HEPATOBLASTOMA IN CELL CULTURE.

Helson L, Helson C
Memorial Sloan-Kettering Cancer Center, New York, NY 10021
In Vitro; 12(4):327-328 1976

We report the establishment of a human cell line, SK-H-MA, derived from a pleural effusion of an infant with disseminated hepatoblastoma. This line has been maintained in vitro for 3 yr. The cells were grown at 37 C in 25 ml plastic flasks containing Eagles MEM supplemented with 20% fetal calf serum, glutamine and non-essential amino acids. They attach to the plastic readily and present morphologically as monolayers of small round cells with occasional giant cells. Chromosome preparation revealed both aneuploid and diploid cell populations. On electron microscopy, they are seen to contain a minimum of ultrastructural features and to lack viral particles. The population doubling time of cells under these conditions was 44 hr, and flasks inoculated with 100,000 cells were confluent by 7 days. After 3 wk, 2 x 10^6 cells inoculated subcutaneously into nude mice developed into 1-cm tumors, which were histologically similar to the primary hepatoblastoma. Conditioned media from 7-day cultures lacked alpha-fetoglobulin, carcinoembryonic antigen, but contained relatively large amounts of B12 binding protein. The cells were found to contain 5'-nucleotidase by both biochemical and cytochemical analyses. Based upon morphological and biochemical characteristics, it is concluded that this is an established hepatoblastoma cell line.

447. CHARACTERIZATION OF A COBALAMIN-BINDING PLASMA PROTEIN FROM A PATIENT WITH HEPATOMA.

Nexo E, Olesen H, Christensen JM, Thomsen J, Kristiansen K
Bispebjerg Hosp., DK-2400 Copenhagen NV, Denmark
Scand J Clin Lab Invest; 35(7):683-690 1975

A cobalamin-binding protein was purified by affinity chromatography from the plasma of a patient with hepatoma and a 10,000-fold increase in plasma cobalamin-binding capacity. The protein behaved as normal transcobalamin I in gel filtration, agar gel electrophoresis, immunoelectrophoresis, precipitation by ammonium sulfate, and cobalamin-binding studies. The protein contained 38% carbohydrate, and the relative molecular mass based on amino acid and carbohydrate analyses was 69,000. The molar absorption coefficient of cyanocobalamin bound to the protein was 36,000 at 362 nm. On amino acid sequencing, 1 amino terminal was found and the first 13 residues were determined as glutathione (Glu)-isoleucine (Ile)-serine (Ser)/cystine-Glu-valine-Ser/Cys-Glu-Glu-asparagin-tyrosine/Ile- arginine-leucine/Ile.

448. A RARE CASE OF MEGALOBLASTIC ANAEMIA CAUSED BY DISTURBANCES IN THE PLASMA COBALAMIN BINDING PROTEINS IN A PATIENT WITH HEPATOCELLULAR CARCINOMA.

Nexo E, Olesen H, Norredam K, Schwartz M
Bispebjerg Hosp., DK-2400 Copenhagen NV, Denmark
Scand J Haematol; 14(5):320-327 1975

A 47-yr-old woman was hospitalized because of hepatomegaly and macrocytic anemia. There was a 2-yr history of wt loss and fatigue. A plum-sized tumor in the right medioaxillary line in the 9th intercostal spaces was shown to be a metastasis from a hepatocellular carcinoma. The patient was given cytostatic treatment and frequent transfusions, but the disease slowly progressed, culminating in death after 11 mo. Hematological investigations showed a low blood hemoglobulin, increased mean corpuscular vol, and extreme increase in serum cobalamin. Plasma transcobalamin (TC) I was increased to 10,000 times the normal conc and plasma TC II was undetectable. Early bone marrow microscopy showed a slight shift of myelopoiesis to the left and no megaloblasts. Marrow examinations later in the disease showed a marrow dominated by immature megaloblasts. Serum albumin, immunoglobulin A and G, and bilirubin conc remained within normal limits and no alpha1-fetoprotein or hepatitis associated antigen were detected in the serum. Liver biopsies taken on 2 occasions showed a hepatocellular carcinoma, grade III; the greater part of the tumor cells contained 1 or more roundish cytoplasmic inclusion bodies filled with opaque

membrane-bound vesicles and/or electron-dense, finely fibrillar material. Some of the tumor cells contained an increased amount of tightly packed, granular, endoplasmic reticulum. The patient had a moderate proteinuria. It is likely that the increased amounts of plasma TC 1 were produced by the tumor cells.

449. BIOCHEMICAL ABNORMALITIES IN SOME HUMAN NEOPLASMS: (1) THE LEUKAEMIAS. (PP. 77-85)

Bertino JR, Allaudeen HS

Scientific Foundations of Oncology. Symington T, Carter RL, ed. London, William Heinemann Medical Books Ltd, 1976.

Discussion is made of biochemical studies directed at elucidating differences between normal and leukemic leukocytes. Particular emphasis is placed upon current knowledge of pyrimidine and purine biosynthesis, as well as RNA and DNA metabolism. Discussion is also made of energy metabolism, leukocyte alkaline phosphatase, vitamin B12 binding protein, asparagine biosynthesis, and 5'-nucleotidase. The most significant finding so far has been that reverse transcriptase may be a DNA polymerase unique to the neoplastic cell. Such a finding suggests, but does not prove, a viral etiology for leukemia. Only 30% of human leukemic cells have been found to contain reverse transcriptase activity, but absence of the enzyme in other cases may be due in part to interference by proteases that can modify its activity. Other biochemical differences between normal and neoplastic cells include the lack of capacity for asparagine biosynthesis in acute lymphocytic leukemia cells compared to normal cells, and the absence or marked decrease in 5'-nucleotidase in chronic lymphocytic leukemia cell membranes compared to normal lymphocyte membranes. (109 refs)

450. HYPERSIALYLATION; A MECHANISM FOR ACCUMULATION OF ACID GLYCOPROTEINS IN MALIGNANCY.

Waxman S, Schreiber C, Helson L

Mount Sinai School of Med. and Memorial Sloan-Kettering, N.Y. N.Y.

Proc Am Assoc Cancer Res; 17:76 1976

A serum B12 binding protein with increased sialic acid content (HB12BP) causing elevations (500 times normal) of serum B12 and unsaturated B12 binding capacity (UB12BC) has been found in hepatoma. We now report another patient with hepatoma with initial near normal UB12BC which increased 400 fold as the disease progressed and then fell 50% with response to chemotherapy. A perfusate of the liver had 5 times more B12 binding protein than the serum and was immunologically the same as serum HB12BP isolated from the previous cases. The perfusate and the serum HB12BP had two isoelectric components (pI 2.8 and 3.2). A cell line derived from hepatoma in another patient with normal UB12BC produced B12 binding protein which contained two isoelectric components (a minor pI 2.9 and a major 3.9), Cell lines derived from normal liver and the Chang hepatoma produced B12 binding proteins similar to that in normal serum (2/3 Transcobalamin II). The hepatoma sera and perfusate had an increase in sialyltransferase. These data suggest that some hepatomas produce increased hypersialylated B12BP (pI 2.8) which is cleared slowly and accumulates in the serum as HB12BP. This may happen to other unsaturated but not saturated sialoglycoproteins in malignancies where elevated sialyltransferase has been reported. (Author Abstract)

451. ABSENCE OF VITAMIN B12-BINDING PROTEINS IN THE HISTAMINE-CONTAINING GASTRIC CARCINOID IN PRAOMYS (MASTOMYS) NATALENSIS.

Nakamura W, Hosoda S

Laboratory of Pathology, Aichi Cancer Center Research Institute

Gann; 67(3):437-439 1976

The transplantable argyrophilic gastric carcinoids producing histamine found in Praomys (Mastomys) natalensis contained no significant amount of vitamin B12-binding proteins. It was also demonstrated that the concentration of vitamin B12-binding proteins in glandular stomach of mastomys was much lower than those in the same tissues of the rat or mouse. (Author Abstract)

C. Vitamin D3 and Calcium Binding Proteins

452. MINIREVIEW: A CALCIUM TRANSPORT SYSTEM FOR MAMMALIAN CELLS.

Hajdu S, Leonard EJ

Lab. Kidney-Electrolyte Metabolism, Nat. Heart Lung Inst., Bethesda, MD

Lif Sci; 17(10):1527-1534 1975

A three-compartment calcium transport system (CTS) located external to the electric membrane and oriented opposite to the cell membrane calcium pump in mammalian systems is described. The three-compartment system includes the actomyosin region, sarcoplasmic reticulum to induce relaxation by removing calcium from the actomyosin region, and a calcium "pot" from which calcium is released into the cells during excitation. The outer boundary of the CTS space contains a CTS layer which restricts calcium ion permeability and creates a zone in which the "full pot" level is about 8-12 mM Ca^{2} 1/liter. Experimental support for this three compartment system is presented initially as an interval tension curve of a rat diaphragm tested after 0, 2.5, 4, and 5 hr of perfusion in calcium free medium. Twitch tension remains maximal with stimuli applied every 2 sec. However, soaking in calcium free medium diminishes the rate at which the "pot" can be refilled. Secondly, the osmotic shock caused by shifting glycerol soaked muscles back into normal extracellular fluid is associated with a sudden increase in the volume of the T-system space with rupture into the adjoining sarcoplasm. These results are best explained by postulating an outside skin such as the CTS layer. Finally, the external location of the CTS layer was determined by the addition of caffeine to an isolated rat diaphragm. A caffeine-sensitive calcium-binding protein was isolated. The amount of calcium-protein complex extracted was less in caffeine-treated than in control muscles. These findings are consistent with the idea that caffeine contraction is caused by a direct action of the caffeine on the CTS layer resulting in a release of calcium and a sudden rise in Ca^{2} 1 in the CTS space.

453. HYPOCALCEMIA IN LEUKEMIA.

McKee LC

Vanderbilt Univ. Sch. Medicine, Nashville, Tenn. 37203

South Med J; 68(7):828-832 1975

In a series of 182 unselected hospital admissions hypocalcemia occurred in 19 patients, including 5 with acute leukemia, 5 with chronic lymphatic leukemia, and 9 with chronic myeloid leukemia. Although hypocalcemia in leukemia can usually be explained on the basis of serum albumin depression, lack of plasma calcium-binding proteins, and poor renal function, the 4 cases reviewed in this paper were unexplained on this basis. Spleen and liver enlargement were present in 3 of the 4 cases; serum calcium levels remained at or below 8 mg/100 ml through the course of treatment in 2

cases, while they returned to normal in 1 treated with large doses of 6-mercaptopurine. The author speculates that inadequate calcium intake, busulfan therapy, intestinal infiltration, or increased bone production secondary to fibrosis of bone marrow in leukemia may have been factors accounting for the observed deficits.

454. REGULATION OF ADENYLATE CYCLASE FROM GLIAL TUMOR CELLS BY CALCIUM AND A CALCIUM-BINDING PROTEIN.

Brostrom MA, Brostrom CO, Breckenridge BM, Wolff DJ
Department of Pharmacology, Rutgers Medical School, College of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854

J Biol Chem; 251(15):4744-4750 1976

A biphasic response to changes in $Ca^{2}+$ concentration was observed for basal and norepinephrine stimulated adenylate cyclase activity in homogenates of C-6 glioma cells. The enzyme was stimulated approximately 40% by low concentrations of free $Ca^{2}+$ (less than or equal to 1 mu M) and inhibited to successively greater extents as free $Ca^{2}+$ concentrations were increased to approximately equal to 100 mu M. $Ca^{2}+$ did not alter the concentration of norepinephrine required for enzyme activation. Homogenates of C-6 cells were separated into particulate and supernatant fractions by centrifugation at 27,000 x g for 20 min. The particulate fraction contained nearly all of the adenylate cyclase activity. This activity was stimulated approximately 40% by the addition of untreated supernatant fraction, by boiled or dialyzed supernatant fraction, and by a homogeneous $Ca^{2}+$ -binding protein (calcium-dependent regulator (CDR)) prepared from brain. Addition of either the supernatant fraction or CDR lowered the $Ca^{2}+$ concentration required for maximal stimulation of the adenylate cyclase. The factor in the supernatant fraction which activated the particulate enzyme was subsequently identified in acrylamide gel electrophoretic studies to be CDR. The amount of CDR required for maximal activation of the enzyme was found to be lowered as the $Ca^{2}+$ concentration in the assay was increased. High amounts of added CDR (100 to 1000 ng) were inhibitory. Use of the nonionic detergent, Lubrol PX , to prepare dispersed adenylate cyclase from the particulate fraction resulted in large losses of activity. The resultant preparation of enzyme contained some CDR which could not be removed by chromatography of the preparation on anion exchange columns. Addition of homogeneous CDR to the assay activated the enzyme several-fold at low $Ca^{2}+$ concentrations. At higher $Ca^{2}+$ concentrations the enzyme was activated fully by the CDR endogenous to the preparation and added $Ca^{2}+$ CDR was inhibitory. (Author Abstract)

455. BINDING OF FLUORESCENT LANTHANIDES TO RAT LIVER MITOCHONDRIAL MEMBRANES AND CALCIUM ION-BINDING PROTEINS.

Mikkelsen RB, Wallach DF
Division of Radiobiology, Tufts-New England Medical Center,171 Harrison Avenue, Boston, Mass. 02111 (U.S.A.)

Biochim Biophys Acta; 433(3):674-683 1976

1. $Tb^{3}+$ binding to mitochondrial membranes can be monitored by enhanced ion fluorescence at 545 nm with excitation at 285 nm. At low protein conc (less than 30 ug/ml) no inner filter effects are observed. 2. This binding is localized at the external surface of the inner membrane and is unaffected by inhibitors of respiration or oxidative phosphorylation. 3. A soluble $Ca^{2}+$ binding protein isolated according to Lehninger, AL (Biochem Biophys Res Commun; 42:312-317 1971) also binds $Tb^{3}+$ with enhanced ion fluorescence upon excitation at 285 nm. The excitation spectrum of the isolated protein and of the intact mitochondria are indicative of an aromatic amino acid at the cation binding site. 4. Further characterization of the $Tb^{3}+$ protein interaction revealed that there is more than one binding site per protein molecule and that these sites are clustered (less than 20 a). Neuraminidase treatment or organic solvent extraction of the protein did not affect fluorescent $Tb^{3}+$ binding. 5. pH dependency studies of $Tb^{3}+$ binding to the isolated protein or intact mitochondria demonstrated the importance of an ionizable group of pK greater than 6. At pH less than 7.5 the amount of $Tb^{3}+$ bound to the isolated protein decreased with increase in pH as monitored by $Tb^{3}+$ fluorescence. With intact mitochondria the opposite occurred with a large increase in $Tb^{3}+$ fluorescence at higher pH. This increase was not observed when the mitochondria were preincubated with antimycin A and rotenone. (Author Abstract)

456. ACTINOMYCIN D INHIBITION OF VITAMIN D3-INDUCED CALCIUM-BINDING PROTEIN (CABP) FORMATION IN CHICK DUODENAL MUCOSA.

Corradino RA, Wasserman RH
Cornell U. New York State Vet. Coll., Ithaca

Arch Biochem Biophys; 126(3):957-960 1968

In 1-d-old chicks fed a rachitogenic diet for 3 wk, and inj iv with actinomycin D (AD; 100 MUg), 2 hr before 1 im inj of vitamin D3 (D3), the D3-induced increase in calcium binding activity (determined 16 hr later) in the duodenal mucosa was inhibited markedly but not completely by AD. Similar results were seen when AD was admin simultaneously with D3. The extent of AD-induced inhibition was dependent on the dose of D3. It is suggested that the effect of AD may be dissipating 16 hr after inj. AD also prevented D3-induced enhancement of calcium absorption.

457. CALCIUM-DEPENDENT REGULATORY PROTEIN OF CYCLIC NUCLEOTIDE METABOLISM IN NORMAL AND TRANSFORMED CHICKEN EMBRYO FIBROBLASTS.

Watterson DM, Van Eldik LJ, Smith RE, Vanaman TC
Dept. Microbiology and Immunology, Duke Univ. Medical Center, Durham, NC 27710

Proc Natl Acad Sci USA; 73(8):2711-2715 1976

The concentration of a calcium-binding protein modulator of 3':5'-cyclic-nucleotide phosphodiesterase activity was increased in chicken embryo fibroblasts upon transformation by Rous sarcoma virus. This modulator protein from fibroblasts, which has roughly the same molecular size, charge, and functional properties as that isolated from chicken brain, comprised approximately 1.32% of the soluble protein in homogenates of fibroblasts infected and transformed by Rous sarcoma virus. In comparison, the modulator comprised approximately 0.30% of the soluble protein in homogenates of normal fibroblasts from confluent cultures and 0.36% of the soluble protein in homogenates of fibroblasts infected with a transformation-defective mutant of Rous sarcoma virus. Modulator levels in normal fibroblasts at subconfluent cell densities were 0.42%-0.76% of the homogenate soluble protein; ie, between that found in confluent normal fibroblasts and in fibroblasts transformed by Rous sarcoma virus. These observations suggest that the modulator protein levels are elevated under conditions in which chicken embryo fibroblasts are undergoing rapid growth and have decreased cyclic AMP levels.

458. THE NATURE, PROPERTIES AND REGULATION OF ADENYL CYCLASE AND PHOSPHODIESTERASE OF ADENOSINE-3', 5'-MONOPHOSPHATE.

Dumler IL, Etinhof RN

I. M. Sechenov Inst. of Evolutionary Physiology and Biochemistry of the USSR Acad. of Sciences, Leningrad, USSR

Vopr Med Khim; 22(2):151-161 1976

Current data on the nature, properties and pathways of regulation of enzymes, particularly in the metabolism of cyclic AMP, are presented. The problem of regulation of the phosphodiesterase activity due to the effect of Ca2+ binding proteins is being studied. (101 refs)

459. CYCLIC NUCLEOTIDE PHOSPHODIESTERASE AND PROTEIN ACTIVATOR IN HUMAN CANCER CELL LINES AND BROWN-PIERCE CARCINOMA. (MEETING ABSTRACT).

Liu YP, Wong V, Chabner BA

NIH, Bethesda, Md 20014

Fed Proc; 36(3):687 1977

Adenosine 3', 5' -monophosphate phosphodiesterase (EC 3.1.4.17) and the activating factor of cyclic nucleotide phosphodiesterase were detected in cultured human lymphoblastic leukemia and retinoblastoma, and in Brown-Pierce (rabbit) carcinoma. The homogenate of lymphoblastic cells contained sufficient activating factor to produce maximal activation of the endogenous phosphodiesterase. The activating factor found in these malignant cells appears to be similar to the calcium binding protein activator of bovine brain phophodiesterase based on the molecular weight obtained from gel filtration, electrophoretic patterns, calcium requirement for the activity and the effect of calcium on the proteolysis. In addition, the tumor derived activator was able to restore the activity of activator-deficient phosphodiesterase from the bovine brain. (Author Abstract)

D. Folate Binding Proteins

460. STUDIES WITH THE FOLATE BINDING PROTEIN IN CHRONIC GRANULOCYTIC LEUKAEMIA CELLS. I. SYNTHESIS AND RELEASE OF BINDER BY CELLS IN SHORT-TERM CULTURE.

De Costa M, Rothenberg SP

Div. Hematology/Oncology, Dept. Medicine, New York Medical Coll., New York, NY 10029

Br J Haematol; 34(4):518-587 1976

Chronic granulocytic leukemia (CGL) cells which contained a high concentration of unsaturated folate binding protein were incubated in suspension culture for 5 hr. Cell samples were periodically assayed for binder: these demonstrated active, energy dependent, synthesis of binder which was inhibited by puromycin, cycloheximide, N-ethylmaleimide, and by incubation at 4 C but not by actinomycin D. Folate binding activity was also demonstrated in the culture medium; this increased with the duration of incubation. This release of binder was inhibited by culturing the cells at 4 C and by the addition of N-ethylmaleimide, but not by actinomycin D, puromycin, or cycloheximide. When the pre- and post-culture cell lysates were saturated with tritiated folic acid (H3PteGlu) and chromatographed on DEAE-agarose, approx half of the bound folate eluted with 0.001 M phosphate buffer at pH 6.0 while the other half eluted with 0.2 M buffer at pH 7.2. The culture medium and plasma from this CGL patient as well as serum from two normal subjects saturated with H3PteGlu primarily contained the acidic binder and much less of the binder eluting with the low molarity buffer. Since a folate binding protein immunochemically similar to the binder in CGL cells has been identified in the serum of non-leukemic subjects, the source of circulating folate binding protein may be the immature granulocyte. (9 refs)

461. THE HETEROGENEITY AND PROPERTIES OF FOLATE BINDING PROTEINS FROM CHRONIC MYELOGENOUS LEUKEMIA CELLS.

Fischer CO, Da-Costa M, Rothenberg SP

Dept. Medicine, New York Medical Coll., 1249 Fifth Ave., New York, N.Y. 10029

Blood; 46(6):855-867 1975

Previous studies have demonstrated that some chronic myelogenous leukemia cells contain a macromolecular binding factor for folic acid. This binder, which previously was believed to be a single factor, has now been resolved into 2 distinct binding proteins. Separation of each binder was obtained by DEAE chromatography of the partially purified lysate of chronic myelogenous leukemia cells. One binder has a molecular wt of 30,000-35,000, and the 2nd binder has a molecular wt of 40,000-45,000. Both proteins bind the mono-, di-, and triglutamates of folic acid, N^{10} -methyl-folate, dihydrofolate, and N^5 -methyltetrahydrofolate. Neither binder has determinants for N^5 -formyltetrahydrofolate or methotrexate. The preferred substrates for both binders appear to be the fully oxidized and partially reduced folates rather than the fully reduced folates. The lower-molecular wt folate binding protein shows reversible binding with partially and fully reduced folates but irreversible binding with oxidized folates. This property suggests that this binder may have some function in the transport and storage of folate. The higher-molecular wt folate binding protein, however, has only slight reversibility of binding with the partially and fully reduced folates; it is therefore more difficult to postulate a physiologic function for this binding factor.

462. FOLIC ACID BINDING PROTEIN IN ACUTE MYELOID LEUKAEMIA.

Gorst DW, Courtis M, Delamore IW

Univ. Manchester and the Royal Infirmary, Manchester, England

J Clin Pathol; 29(1):60-62 1976

Folic acid binding protein (FABP) was estimated in 94 normal control subjects, 18 patients with untreated acute myeloid leukemia (AML), 18 folate-deficient patients, six patients in the terminal blastic phase of chronic myeloid leukemia (CML), and two patients with untreated CML. FABP was estimated using tritiated pteroylmonoglutamates to saturate the binders. Folate-deficient patients had a mean level of 139 pg/ml FABP, in comparison with 76 pg/ml in normal subjects. The mean value for the AML patients was 338 pg/ml, and for CML patients was 179 pg/ml. AML patients with exceptionally large monocytic components showed especially high levels of FABP. The two patients with untreated CML had normal levels. The finding high levels of a folate binding protein (normally present in very small amounts) in the serum of patients with untreated AML is unexpected.

463. FOLIC ACID BINDING PROTEIN IN NORMAL SUBJECTS, FOLIC ACID DEFICIENCY AND ACUTE MYELOID LEUKAEMIA.

Gorst DW, Courtis M, Delamore IW

Manchester Royal Infirmary, Manchester, England

Br J Haematol; 33(4):608 1976

Folic acid binding protein (FABP) was measured in 95 normal subjects: the levels ranged from 20 to 360 pico(p) g/ml; 85% fell within the range of 20 to 150 pg/ml. The distribution bore no simple relationship to a normal distribution. Six subjects in excess of 300 pg/ml were studied. Pure preparations were made of polymorphs, monocytes and lymphocytes and the lysates were assayed for FABP. High levels,

paralleling those in the serum, were found in all polymorph lysates; no measurable FABP was detectable in any other fraction. The molecular wt of the binder is approximately 40,000.

464. FURTHER CHARACTERIZATION OF FOLATE BINDING PROTEINS IN LIVER (MEETING ABSTRACT).

Zamierowski M, Wagner C
VA Hosp and Vanderbilt Univ, Nashville, Tn 37203
Fed Proc; 35(3):581 1976

We have previously shown by i.p injection of (G3H)-folic acid into rats 24 hr before sacrifice that three separate folate binding proteins are present in rat liver cytosol and one is present in the nuclear-rich pellet of the liver (BBRC 60, 81, 1974). These bound forms of folate are primarily polyglutamates. Further studies have now shown that the folate binding protein in the nuclear-rich pellet is contained in the mitochondria which are present in this cell fraction. When rats are sacrificed 30 min after injection, most of the bound folate was present in the microsomal fraction of the cell. This was identified as non-metabolized plasma membrane-bound folic acid. With increasing times after injection both the amount and binding of folate decreased in the microsomes and increased in the crude nuclear and cytosol fractions. In folate deficient animals 24 hr after injection there is increased binding of labeled folate to only one of the binding proteins in the cytosol. Very little unbound folate was found in the livers of folate deficient animals. These studies suggest a storage role for certain of the folate binding proteins of rat liver. (Author Abstract)

465. LACK OF INFLUENCE OF ORAL CONTRACEPTIVES ON SERUM FOLATE, HEMATOLOGIC VALUES, AND UTERINE CERVICAL CYTOLOGY.

Ross CE, Stone MK, Reagan JW, Wentz WB, Kellermeyer RW
Depts. Medicine, Pathology, and Obstetrics-Gynecology, Case Western Reserve Univ., Univ. Hosps. Cleveland, Cleveland, OH 44106
Semin Hematol; 13(3):233-238 1976

Serum folate (free, total, and that bound to folate-binding proteins) was correlated with a cytological examination of the uterine cervical epithelium, diet, and standard hematological values in order to ascertain the influence of oral contraceptives on these factors. A total of 172 randomly selected women, 19-35 yr old, were studied. Those women who had never taken oral contraceptives or had not taken this medication for 9 mo were placed in the control group (77), and those taking oral contraceptives in excess of 9 mo were placed in the study group (95 women). None of the women in the study or control groups had erythrocyte macrocytosis or serum folate deficiency. There was no difference in the mean hematocrit or Hb value or in the mean corpuscular volume of erythrocytes for the two groups. No significant differences between the two groups were found for mean serum folate levels (either total or free); serum folate could not be correlated with either length of time the risk group had been on oral contraceptives, the general type of contraceptive used, or income. Two women, one in the control and one in the study group, had megaloblastic cervical cytology, but normal hematological and serum folate values. There were no cases of megaloblastic anemia. When this anemia occurs secondary to folate deficiency associated with oral contraceptives, as found in other studies, it may be related to a genetic or acquired susceptibility for malabsorption of folic acid that is manifested by oral contraceptives.

466. THE ISOLATION OF THE FOLATE BINDING PROTEIN FROM COMMERCIALLY PURIFIED BOVINE BETA LACTOGLOBULIN.

Waxman S, Schreiber C
Mt. Sinai Sch. Medicine, City Univ. New York, N.Y. 10029
FEBS Lett; 55(1):128-130 1975

The isolation of folate-binding protein from the beta lactoglobulin fraction of cow's milk in sufficient quantity to enable its preliminary characterization is described. Folate binding protein was clearly separated from beta lactoglobulin and purified 150-fold by elution from the DEAE column with the early effluent at a near zero salt conc. No binding of titrated pteroylglutamic acid ^{3} HPGA was observed with the protein fractions associated with the A or B alleles of the beta lactoglobulin. There were two protein bands in the upper 1/3 of the acrylamide gel both of which bound ^{3} HPGA, whereas beta lactoglobulin was found in the region of the tracker dye without a corresponding radioactive peak. The molecular wt of ^{3} HPGA-labeled folate binding protein was 36,000. There were three peaks of folate-binding protein with a pI of 7.2, 7.8, and 8.6, all of which were immunologically distinct from beta lactoglobulin. Antibody to beta lactoglobulin did not block ^{3} HPGA binding or increase the size of ^{3} HPGA bound to impure beta lactoglobulin. ^{3} HPGA bound to folate-binding protein was not available to the HeLa cell monolayer. There are similarities between the folate-binding protein of cow's milk and the folate binding protein obtained from the milks of goat and human. It is clear that folates bound to this protein are unavailable for cellular uptake and could significantly affect a folate-dependent cell or organism.

467. FOLATE TRANSPORT IN LACTOBACILLUS CASEI: SOLUBILIZATION AND GENERAL PROPERTIES OF THE BINDING PROTEIN.

Henderson GB, Zevely EM, Huennekens FM
Scripps Clinic and Res. Foundation, 476 Prospect St., La Jolla, CA 92037
Biochem Biophys Res Commun; 68(3):712-717 1976

The authors describe the general properties of a folate-binding protein, a component of the transport system in cells. Intact cells of Lactobacillus casei grown in a medium containing a low level (5 nanomolar) of folate have the capacity to bind (at 4 C) appreciable quantities (0.35 nanomoles/10^{10} cells) of the vitamin. Folate binding is rapid, saturable (Kd = 36 nanomolar), insensitive to sulfhydryl reagents, and has a broad pH optimum. A folate-binding protein was solubilized in high yield by sonic disruption of lysozyme-treated cells in the presence of ^{3} Hfolate and Triton X-100. The protein-folate-Triton complex (MW 230,000) was stable to dialysis but dissociated upon heating (50% loss of bound folate after 5 min at 49 C). The binding protein may function as the carrier of folate during its transport into the cells.

VII. DRUG BINDING PROTEINS

468. METHOTREXATE BINDING PROTEINS IN L1210 LYMPHOCYTE PLASMA MEMBRANES (MEETING ABSTRACT).

McCormick JI, Rader JI, Freisheim JH
Dept of Biological Chemistry, Univ of Cincinnati, Coll of Medicine, Cincinnati, OH 45267
Fed Proc; 36(3):804 1977

The folate antagonist, Methotrexate (MTX) is transported into L1210 cells by an active carrier-mediated process. A rapid procedure for the extraction of plasma membrane proteins from L1210 cells has been applied to the isolation of membrane associated MTX-binding proteins. The procedure involves incubation of intact L1210 cells in phosphate-

buffered saline, pH 7.4, containing 0.01% Triton X-100 (w/v). Under these conditions the transport of MTX ceases and increasing amounts of protein are released from the cell as the incubation proceeds. The proteins released have been shown to be mainly plasma-membrane derived by assay of membrane marker enzymes and by SDS gel electrophoresis. The gel electrophoresis pattern of proteins solubilized is comparable to that obtained after solubilization of an isolated plasma membrane pellet. MTX-binding proteins have been isolated by use of a Sepharose-MTX affinity column. Following the appearance of non-adsorbed material in the effluent, a fraction is isolated by elution with MTX which contains 3 major proteins species with molecular weights of 67,000, 63,000 and 56,000, as determined by SDS gel electrophoresis. This fraction has been shown to bind (^{3}H)MTX, based on gel filtration studies. These 3 polypeptides appear to be subunits of a high molecular weight complex involved in MTX binding. (Author Abstract)

469. LIGAND BINDING ASSAY OF 3H-METHOTREXATE AND ITS APPLICATION TO PLASMA LEVEL MEASUREMENTS (MEETING ABSTRACT).

Klotz U, Antonin KH, Wilmanns W, Bieck PR
Dr. Margarete Fischer-Bosch-Inst. Clinical Pharmacology, D 7000 Stuttgart 50 Auerbachstr, 112, W. Germany
Arch Pharmacol; 294(Suppl):R10 1976

A specific and sensitive ligand binding assay using H3-methotrexate (MTX) that is similar to another recently published method is described. A binding protein (BP) was prepared from guinea pig liver homogenate by ammonium sulfate fractionation and gel filtration. For optimal binding of 3H-MTX the addition of mercaptoethanol and NADPH was necessary. Without these compounds the percentage of binding was reduced by a factor of two. Binding was proportional to the concentration of BP and had similar pH-optima (6.0 to 6.5) in citrate-, tris-, and phosphate buffer: in phosphate buffer, binding was slightly higher. The binding of 3H-MTX was constant over a period of 5 to 30 min but dependent on the incubation temperature: between 20C and 37C it decreased linearly and at 4C MTX was maximally bound. Binding of 3H-MTX was not influenced by physiological concentrations of tetrahydrofolate, dihydrofolate, folic acid, and leucovorin. For measuring plasma levels of MTX in patients, the competitive binding of 3H-MTX (15Ci/mmol) was measured in phosphate buffer (pH 6.0) containing 8 microM mercaptoethanol and 100 microM NADPH by incubating 50 microl plasma samples with 15 microg BP for 15 min in an ice-bath. Bound and free 3H-MTX was separated by the addition of a charcoal suspension and the free 3H-MTX was measured after centrifugation in the supernatant by scintillation counting. The calibration curve for MTX was linear in the range of 5-4000 nanog/ml. The method was applied to plasma level monitoring in one patient, who received in therapeutic intervals five different doses of MTX by a 6 hr infusion. (no refs)

470. STRUCTURE ACTIVITY RELATIONSHIPS IN A SERIES OF ANTINEOPLASTIC DINITROPHENYL AZIRIDINES-PROTEIN BINDING STUDIES.

Hickman JA
Chester Beatty Res. Inst., Inst. Cancer Res., R. Cancer Hosp.,
Biochem Pharmacol; 23(20):2833-2838 1974

In a study of the reversible binding of a series of antineoplastic 2,4-dinitrophenylaziridines (CB 1954 and the corresponding mono- and di-methyl- and ethyl-substituted amides) to bovine serum albumin, methods of gel filtration and continuous diafiltration were used to estimate the amount bound and the reversibility of binding. The compounds were shown to be weakly but reversibly bound; the extent of binding correlated well with biological activity and molecular structure. Results substantiate previous work and suggest that a receptor site may exist for the amide CB 1954 and that the ability to bind to such a site is essential for antitumor activity; efforts to characterize this site are underway. (11 refs)

471. MICROTUBULES AS DRUG RECEPTORS: PHARMACOLOGICAL PROPERTIES OF MICROTUBULE PROTEIN.

Wilson L
Dept. Pharmacol., Stanford Univ. Sch. Med., Calif.
Ann NY Acad Sci; 253:213-231 1975

The biochemical mechanisms of action of colchicine (C), podophyllotoxin (P) and the vinca alkaloids on microtubule function are reviewed. The 1st-order decay process of C-binding is stressed, and the biochemical nature of this decay process is explained. Although the antimitotic properties of P seem indistinguishable from those of C and these drugs share the same binding site on tubulin, the mechanism of the P-binding reaction is substantially different from that of the C-binding reaction (the former binds more rapidly and is rapidly reversible). Vinblastine (VLB) precipitates tubulin in a concn-dependent manner. This effect, however, is nonspecific. It has been found that VLB could precipitate a large number of acidic proteins (such as muscle actin and double-stranded DNA). Characterization of the high-affinity VLB binding sites on chick embryo brain tubulin, the VLB-binding reaction, and how the vinca alkaloids disrupt microtubules are also discussed. Data suggest that vinca alkaloids need to bind to less than 1% of the total tubulin in order to disrupt microtubule formation half-maximally. It is postulated that C, P, and the vinca alkaloids work by the general mechanism of "poisoning" microtubule assembly. (43 refs)

472. VINCA ALKALOIDS AND COLCHICINE.

Creasey WA
No affiliation given
Handbuch fuer Experimentelle Pharmakologie: Antineoplastic and Immunosuppressive Agents; (Springer, New York) 38(2):670-694 1974-1975

Investigations of the vinca alkaloids and colchicine derivatives are reviewed. Although chemically distinct, these compounds constitute a single class of agents in view of their similar properties, particularly their ability to arrest cell division in metaphase. Structure-activity relationships are outlined, and evidence for reversible spindle dissolution as the mechanism of metaphase arrest is presented. Reports of experimental tumor inhibition by colchicine, colcemid, the vinca alkaloids, and the podophyllotoxin derivatives are cited, together with data on neurotoxicity and other biologic effects. These agents have been shown to cause the disappearance of microtubules in HeLa cells and human leukocytes and platelets, and are associated with specific cellular binding proteins; interference with phagocytosis, morphology, and motility, as well as neurologic effects, are all potential consequences of alterations in the microtubule system. Data on inhibition of nucleic acid and protein biosynthesis and of amino acid uptake are summarized, together with results of pharmacologic studies in animals and man. The vinca alkaloids are established agents in the treatment of lymphomas (especially Hodgkin's disease), acute leukemia, choriocarcinoma, and embryonal or mesenchymal carcinomas such as Wilms' tumor, neuroblastoma, rhabdomyosarcoma, and testicular carcinoma. Responses have also been reported in mycosis fungoides, Letterer-Siwe disease, oat cell carcinoma of the lung, breast carcinoma, the blastic crisis stage of chronic myelocytic leukemia, and some central nervous system neoplasms. Vinblastine is more effective than vincristine in the treatment of advanced Hodgkin's disease; whereas, vincristine

is more active in lymphosarcoma, reticulum cell sarcoma, and acute lymphoblastic leukemia. Leukopenia is the most prominent side effect of vinblastine but is relatively uncommon with vincristine. Vincristine produces a range of neurologic and neuromuscular symptoms (the most consistent being depression of the Achilles tendon reflex). A rare side effect of vincristine is hyponatremia, apparently resulting from inappropriate antidiuretic hormone secretion. (195 refs)

473. PROPERTIES OF COLCHICINE BINDING PROTEIN FROM CHICK EMBRYO BRAIN.

Wilson L
Stanford U. Sch. Med., Calif.
Biochemistry (Washington); 9(25):4999-5007 1970

The binding activity of the colchicine (C)-binding protein (considered to be a microtubule subunit) from chick embryo brain homogenates reached a max at pH 6.7-6.8. A shift in pH or in ionic strength influenced the activity indirectly by increasing the protein inactivation constant, not by influencing the protein association reaction. Vinblastine (VLB) and vincristine (VCR), which like C can disrupt microtubule systems, stabilized the C-binding activity in direct proportion to the logarithm of their concn. (in the concn ranges which produced disruptive effects on microtubules), but did not influence the protein-C association reaction. Unlike VLB and VCR, podophyllotoxin inhibited the binding of C to the protein, but when added to preformed protein-C complex, it increased the stability of the binding activity.

474. EFFECT OF STRUCTURAL ANALOGUES OF NICOTINIC ACID ON THE MITOBLASTIC ACTIVITY OF COLCHICINE.

Deysson G, Delcourt A
Sch. Pharm. Cell. Biol. Lab., Paris
C R Soc Biol (Paris); 164(12):2440-2443 1970

In Allium sativum root tips, colchicine (C; 7.5 x 10-5 M/ml)-induced mitotic arrest was potentiated by addition of 6-aminonicotinamide (5 x 10-6 and 10-5 M/ml). The results support the hypothesis that C inhibits mitosis by acting indirectly on dehydrogenases by binding protein used in spindle formation. The mitoblastic activity of C was not affected by 3-acetylpyridine (another analog of nicotinic acid).

475. CYTOSTATIC DRUGS, MECHANISM OF ACTION. (PP. 236-245)

Drews J
Molecular Base of Malignancy New Clinical and Therapeutic Evidence Selected Papers from an International Symposium Deutsch, E. Moser K, Rainer H, Stacher A, ed., Stuttgart, Georg Thieme Publishers, 1976.

Drugs available for cancer chemotherapy may be classed as alkylating agents, antimetabolites, alkaloids, hormones, antibiotics, or miscellaneous synthetics. Brief descriptions of their biochemical mechanisms of action are presented. The nonselective action of early alkylating agents (nitrogen mustards) stimulated the search for a substance which would be transported to tumor cells without harming the host and would then be metabolized to its toxic potential. Cyclophosphamide, for example, must be metabolized to generate a highly reactive cytotoxic intermediate which tumor cells cannot convert to atoxic metabolites. The antimetabolites aminopterin and methotrexate are structural analogues to folic acid, and 5-fluorouracil is an analog of uracil. These drugs block the formation of DNA precursors, resulting in a decrease in DNA. 6-Mercaptopurine also interferes with DNA and RNA synthesis by leading to a decrease in purine nucleotide precursors. The vinca alkaloids vinblastine and vincristine cause metaphase arrest in dividing cells by binding to microtubule protein. The glucocorticoid hormones prednisone and prednisolone exert a destructive influence on lymphoid cells by binding to tissue steroid receptors which may be present. The antibiotics actinomycin D, daunomycin, and adriamycin interact with DNA by intercalation. They cause a local unwinding of the helix and inhibit DNA-dependent RNA synthesis. Other antibiotics which use different mechanisms to inhibit DNA and RNA synthesis include mithramycin, chromomycin, olivomycin, streptozotocin, and bleomycin. The selectivity of the various cytotoxic agents might be explained by differences in kinetics of malignant and nonmalignant cells, by quantitative differences in these cells' use of particular metabolic pathways, or in the uptake of the agents. Drug combinations are used in an attempt to overcome narrow margins of selectivity of single drugs, but the reasons for the different degrees of effectiveness of various combinations are not yet understood in biochemical terms. (40 refs)

476. RESISTANCE TO ANTITUMOR AGENTS. (PP. 641-654)

Harrap KR
Scientific Foundations of Oncology. Symington T, Carter RL, ed. Chicago, Year Book Medical Publishers Inc, 1976.

An extensive review of cellular resistance to antitumor agents is presented. Twelve mechanisms of induction of both intrinsic and acquired resistance are discussed. These include: impaired uptake; deletion of activating enzymes; increased levels of drug-degrading enzymes; increases in levels of target enzymes, such that the latter are saturated only at drug concentrations which generate prohibitive systemic toxicity; modification of the tertiary structure of the target enzymes such that inhibitor-binding, but not substrate-binding, is precluded; adoption of alternative metabolic pathways bypassing the drug-imposed block; enhanced levels of a normal metabolite which competes with the drug for the target site; repair of damaged DNA; deletion of specific binding proteins necessary for the cytotoxic activity of the drug; modification of tumor cell antigenicity; drug-induced immunosuppression; and kinetic insensitivity. Drug resistance is likely to result from some combination of the above mechanisms. Agents discussed include methotrexate, 5-fluorouracil, azacytidine, cytosine arabinoside, 6-mercaptopurine, 6-thioguanine, cyclophosphamide, chlorambucil, nitrogen mustard, glucocorticoids, actinomycin D, daunomycin, mithramycin, and the Vinca alkaloids. (152 refs)

VIII. OTHER SERUM AND INTRACELLULAR BINDING PROTEINS

477. STRUCTURAL, FUNCTIONAL, AND IDIOTYPIC CHARACTERISTICS OF A PHOSPHORYLCHOLINE-BINDING IGA MYELOMA PROTEIN OF C57BL/KA ALLOTYPE.

Claflin JL, Rudikoff S, Potter M, Davie JM
Washington Univ. Sch. Med., St. Louis, Mo.
J Exp Med; 141(3):608-619 1975

Plasmacytoma CBPC 2 was induced in CB-20 mice (a strain congenic to BALB/c, but differing by carrying the A15 allotypic determinant found in C57CL/ka mice) to characterize an IgA protein produced by the myeloma which binds phosphorylcholine (PC). The IgA PC-binding protein has different IgCH allotypic determinants from those of BALB/c mice. Sequence and isoelectric point analysis revealed the light chain of the CBPC 2 protein to be the same as the light chain of T15, a protein which binds PC and is produced by myelomas in BALB/c mice. The heavy chains of the T15 and CBPC myeloma proteins differ by two amino acids at positions 14 and 16. Both proteins were found to have similar

specificities for acetylcholine , choline , glycerophosphorylcholine and PC. A binding site associated idiotypic determinant present on the T15 protein was also found on the CBPC 2 protein. The non-binding site idiotypic determinant was not present in CBPC 2 protein. This is similar to findings in normal and induced C57BL/6 anti-PC antibody. It is concluded that the structural differences in these proteins relate to allotypic characteristics in the CH region or to a difference in a variable region outside the combing site. Thus, the combining site is similar in structure in two strains of mice that differ considerably genetically.

478. SIZE DIFFERENCES AMONG IMMUNOGLOBULIN HEAVY CHAINS FROM PHOSPHORYLCHOLINE-BINDING PROTEINS.

Rudikoff S, Potter M
Lab. Cell Biology, Natl. Cancer Inst., Bethesda, MD 20014
Proc Natl Acad Sci USA; 73(6):2109-2112 1976

An approach to the problem of determining the mechanism(s) of immunoglobulin diversity, which has long been a subject of controversy, was reported. The entire sequences of the heavy chain variable regions of M167 and TEPC 15 (phosphorylcholine-binding myeloma proteins of BALB/c origin) were determined. These sequences were compared with the phosphorylcholine-binding protein M603. T15 differs from M603 at four positions, all of which are located in antigen-binding complementarity regions. M167, in addition to having differences in the complementarity regions, also had five substitutions in the conserved framework portion of the variable region when compared to T15 and M603. Each of the three proteins had a different length in the third complementarity region. It is unlikely that complementarity regions of different lengths associated with similar framework regions could be generated by proposed mechanisms of somatic mutation generally limited to point mutations. These products are more likely directly encoded by different structural germ-line genes.

479. UNIFORMITY IN THE CLONAL REPERTOIRE FOR THE IMMUNE RESPONSE TO PHOSPHORYLCHOLINE IN MICE.

Claflin JL
Dept Microbiology, Univ. Michigan, 6643 Medical Sci. Building II, Ann Arbor, MI 48109
Eur J Immunol; 6(10):669-674 1976

A comparison of the clonal nature of the immune response to phosphorylcholine (PC) was made in nine different inbred mouse strains. The strains included BALB/c, C58, C57BL/6, DBA/2, A, AKR, and CE. Quantitative idiotypic analysis showed that anti-PC antibodies from each strain were composed of antibodies bearing binding-site idiotypic determinants indistinguishable from two different BALB/c myeloma proteins, T15 and M511. Idiotypic determinants of two other PC-binding proteins, M167 and M603, were not detected. Isoelectric focusing of the light (L) chains verified the presence of antibodies similar to T15 and M511 in each strain and indicated the presence of two additional antibodies, one of which has an L chain that cofocuses with M603. Fractionation of anti-Pc antibody with anti-idiotypic antibody showed that immunoglobulins bearing T15 and M511 idiotypic determinants are separate and contain L chains that are uniform and resemble those of T15 and M511, respectively. Thus, these mice, which differ genetically at multiple loci including the heavy chain allotype complex locus, possess, at least in part, an equivalent set of clonotypes specific for PC. This indicates that the genes encoding these antibodies must be contained in the germ line. (40 refs)

480. PRIMARY STRUCTURAL DIFFERENCES IN MYELOMA PROTEINS THAT BIND THE SAME HAPTENS.

Potter M, Rudikoff S, Vrana M, Rao DN, Mushinski EB
NCI, Lab. Cell Biology, Bethesda, MD 20014
Cold Spring Harbor Symp Quant Biol; 41:661-666 1977

Amino acid sequences of antigen-binding myeloma proteins of BALB/c origin with a specificity for phosphorylcholine (PC), inulin and beta-6 D-galactan are presented. The findings indicate that rabbit antisera recognize cross-specific idiotypes common to galactan-binding myeloma proteins and antigalactan antibodies induced in BALB/c mice. It was not possible to determine whether the differences in the three inulin-binding protein variable heavy-chain (VH) sequences are germ-line-carried or somatic mutations. The fact that a series of kappa-type myeloma proteins can be retrieved randomly indicates that the cells producing these specificities are expressed regularly and that they are a susceptible population for neoplastic transformation. Similarities in the primary structure of proteins within a group indicate that specific binding activities in the mouse depend upon activation of specific variable light chain (VL) and VH genes. The V-region sequences provide some insights into the genetic control and differentiation of immunoglobulin V genes, whose activation may depend upon enzymes specific for individual or sets of genes. (38 Refs)

481. HEAVY-CHAIN VARIABLE-REGION SEQUENCE FROM AN INULIN-BINDING MYELOMA PROTEIN.

Vrana M, Rudikoff S, Potter M
Lab. Cell Biology, NCI, NIH, Bethesda, MD 20014
Biochemistry (Washington); 16(6):1170-1175 1977

As part of a study of the antigenic determinants of myeloma proteins, the structure of five inulin-binding proteins is being studied. The entire variable-region sequence of the heavy chain from ABE-47N, a BALB/c inulin-binding mouse myeloma protein, was determined. This protein is unusual in that the third complementarity region (H3) is extremely short, consisting of at the most three and probably only one amino acid. A comparison of the heavy-chain hypervariable regions from mouse, human, and rabbit proteins shows that the variability in length of H3 is greater than that seen in the first or second hypervariable regions. This variability in H3 length suggests a specialized function for this region. Compared to H3 of other species, the H3 from these mice is very short. The size variations of H3 may be important in the generation of functional antibody diversity. (30 Refs)

482. INTERACTIONS OF ACTIN, MYOSIN, AND AN ACTIN-BINDING PROTEIN OF RABBIT PULMONARY MACROPHAGES. III. EFFECTS OF CYTOCHALASIN B.

Hartwig JH, Stossel TP
Medical Oncology Unit, Massachusetts General Hosp., Boston, MA 02114
J Cell Biol; 71(1):295-303 1976

The effects of cytochalasin B on the assembly and gelation of actin ir macrophage extracts was investigated. Low concentrations (10^{-7} M or less) of cytochalasin B reversibly inhibited the temperature-dependent gelation of actin by an actin-binding protein. The cytochalasin B concentrations which max inhibit actin gel formation were 10-x lower than the concentrations which maximally impair phagocytosis by intact macrophages. Cytochalasin B also prevented the polymerization of monomeric actin in sucrose extracts of macrophages in the absence but not the presence of 0.1 M KCl. Cytochalasin B (10^{-6} M) dissolved macrophage extract gels and gels comprised of purified actin and actin-binding protein by dissociating actin-binding protein from actin filaments. This concentration of cytochalasin B, however, did not

depolymerize the actin filaments. These results confirm previous findings that low concentrations of cytochalasin B do not markedly influence the viscosity, morphology, or function of polymerized muscle or cytoplasmin actins.

483. INTERACTIONS OF ACTIN, MYOSIN, AND AN ACTIN-BINDING PROTEIN OF CHRONIC MYELOGENOUS LEUKEMIA LEUKOCYTES.

Boxer LA, Stossel TP
Univ. Indiana Sch. Medicine, Indianapolis, IN 46202
J Clin Invest; 57(4):964-976 1976

Actin, myosin, and a high molecular weight actin-binding protein were purified from chronic myelogenous leukemia (CML) leukocytes. The cytoplasmic extracts prepared with ice-cold 0.34-M sucrose solutions containing Mg^2 1-ATP, dithiothreitol, and EDTA at pH 7.0 underwent rapid gelation when warmed to 25 C. Initially, the gel could be liquified by cooling to ice-bath temperature. With time, warmed cytoplasmic extract gels shrunk into aggregates. The following findings indicated that CML leukocyte actin-binding protein promoted the temperature-dependent gelation of actin in the cytoplasmic extracts, and that CML leukocyte myosin was involved in the contraction of the actin gels: (a) Cytoplasmic extract gels initially contained actin as their major polypeptide component and consisted of tangled thin filaments; (b) Contracted aggregates of cytoplasmic extract gels contained large quantities of myosin as well as actin; (c) Purified actin-binding protein underwent a temperature-dependent, reversible aggregation and caused low concentrations of purified muscle or CML leukocyte actins to gel in sucrose solutions; (d) The gels formed from purified actin plus purified actin-binding protein slowly contracted in the presence but not in the absence of purified CML leukocyte myosin; (e) Rabbit antiserum against purified CML leukocyte actin-binding protein, but not against purified CML leukocyte myosin, inhibited the gelation of warmed CML leukocyte extracts. Antiserum against CML leukocyte myosin had no effect on the gelation of CML leukocyte extracts but partially curtailed the contraction of the CML leukocyte extract gels and of gels formed from purified CML leukocyte actin-binding protein plus rabbit skeletal muscle actin. These findings are consistent with the idea that reversible polymerization of actin in neutrophils is involved in several aspects of neutrophil behavior, and provide insight into the mechanism of neutrophil filament assembly and movement.

484. BINDING BY PROTEINS OF THE RAT LIVER CYTOSOL OF COMPOUNDS DERIVED IN VIVO FROM THE HAEM PRECURSOR 5-AMINOLAEVULINIC ACID.

Ketterer B, Srai KS, Tipping E
Middlesex Hosp. Medical Sch., London W1P 5PR, England
Biochem Soc Trans; 4(2):202-204 1976

In order to observe the presence or absence of binding proteins during heme-biosynthesis, intracellular transfer, and catabolism, the heme-precursor 5-amino G-H3 levulinic acid was administered to rats; protein-bound radioactivity in the soluble supernatant fraction from the liver analysed by gel filtration. Labeling periods of 10 and 60 min were used. At 60 min, five labeled compounds were separated. Heme- was crystallized in the first three. Experiments with 5-amino G-H3 levulinic acid and CoCl2 to determine the relationship between the binding proteins present and heme-catabolism supported the hypothesis that the proteins bound a product of heme-oxygenase activity in keeping with the presence of another protein which bound bilirubin.

485. HEME-BINDING PROTEINS OF THE RAT LIVER CYTOSOL.

Ketterer B, Srai KS, Christodoulides L
S.A. Couriauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W1p5pr(U.K.)
Biochim Biophys Acta; 428(3):683-689 1976

Gel filtration of soluble supernatant fraction obtained from livers of rats 10 min after an injection of the heme precursor 5-amino(^{3}H)laevulinic acid shows the presence of a major radioactive fraction which upon gel filtration is similar to elution volume to ligandin. 20 min after administration of the precursor four previously minor components also come into prominence. This pattern is a characteristic of in vivo binding since a different elution pattern is obtained if soluble supernatant fraction from rat liver is labelled in vitro by incubation either with (^{3}H)heme-labelled mitochondria, (^{3}H)heme-labelled microsomes or with (^{3}H)hemin. These results are discussed with particular reference to ligandin. (Author Abstract)

486. THYROID HORMONE ACTION IN CELL CULTURE: DEMONSTRATION OF NUCLEAR RECEPTORS IN INTACT CELLS AND ISOLATED NUCLEI (MEETING ABSTRACT).

Samuels HH, Tsai JS
New York U. Med. Ctr., N.Y.
Proc Natl Acad Sci USA; 70(12):3488-3492 1973

The binding of (125I)triiodothyronine and purified (125I)thyroxine to various subcellular components of GH1 cells after incubation with intact cells in serum-free medium was examined. High-affinity, low-capacity binding sites for the hormones were noted in nuclear but not in mitochondrial or cytosol fractions. Chromatographic analysis of the bound nuclear radioactivity from cells incubated with (125I)thyroxine showed 97% thyroxine, 1% iodide, and 1% triiodothyronine. Apparent equilibrium dissociation constants were 20pM for triiodothyronine and 260 pM for thyroxine. The maximal binding capacity was identical for both hormones, with about 5000 sites/cell nucleus. Binding with (125I) thyroxine was competitively inhibited by triiodothyronine. It is suggested that triiodothyronine and thyroxine interact with identical nuclear receptors, and that conversion of thyroxine to triiodothyronine may not be a prerequi site for biological activity. When (125I)triiodothyronine was incubated directly with isolated nuclei, similar high-affinity, low-capacity nuclear binding sites were demonstrated. Increasing the concentrations of nonradioactive triiodothyronine caused a subsequent increase in binding when (125I)thiiodothyronine was then incubated directly with isolated nuclei, suggesting that nuclear receptors are not fixed, but increase after exposure of intact cells to hormone. This increase in nuclear receptor content may result from the transfer of an unstable cytosol receptor to the nucleus. It is suggested that these high-affinity nuclear binding sites are important in mediating the action of thyroid hormones. The localization of the high-affinity binding to the cell nucleus supports the theory that thyroid hormones modulate gene activity.

487. BINDING OF 25-HYDROXYCHOLESTEROL AND CHOLESTEROL TO DIFFERENT CYTOPLASMIC PROTEINS.

Kandutsch AA, Chen HW, Shown EP
Jackson Lab., Bar Harbor, ME 04609
Proc Natl Acad Sci USA; 74(6):2500-2503 1977

Studies were carried out to determine whether or not oxygenated derivatives of cholesterol (eg, 25-hydroxycholesterol) that specifically suppress the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (CA) reductase mevalonate:NADP+ oxidoreductase (CoA-acylating), EC

1.1.1.34 bind to a soluble component of the cytoplasm different from that which binds the nonsuppressor, cholesterol. Density gradient fractionation of the cytosolic fraction isolated from L cell cultures that had been incubated with low concentrations of 25-hydroxy26,27-H3cholesterol or 1,2-H3cholesterol provided evidence for the existence of at least two different sterol-binding proteins. Bound cholesterol sedimented in a sucrose density gradient as two or more broad bands with coefficients of approx 9 S and 21 S. Two relatively narrow bands of bound 25-hydroxycholesterol had sedimentation coefficients of 5 S and 8 S. Preincubation of the cells with a relatively high concentration of unlabeled 25-hydroxycholesterol altered the banding pattern of the 25-hydroxyH3cholesterol taken up during a subsequent incubation period by decreasing the size of the major (8S) band. Under these conditions, cholesterol did not affect the banding pattern of 25-hydroxyH3cholesterol. The density gradient banding pattern of bound H3cholesterol was only slightly affected by preincubating the cells with unlabeled cholesterol or 25-hydroxycholesterol. Both sterols appeared to be bound to proteins because the bound sterols were eliminated from cytosol that had been heated at 100 C, and their sedimentation coefficients were altered by proteolysis. (Author abstract) (27 Refs)

488. SPECIFIC SPERMINE BINDING BY PROSTATE PROTEIN. (MEETING ABSTRACT).

Liang T, Liao S
Univ Chicago, Chicago, IL 60637
Fed Proc; 36(3):388 1977

We have used radioactive polyamines to determine whether polyamine-specific binding proteins are present in the cytosol fraction of rat ventral prostate. By gradient centrifugation, (^{3}H)spermine was found to form a 3S complex with protein(s) that could be purified by Sephadex gel filtration and DEAE-cellulose column chromatography. The formation of the 3S complex was not significant when radioactive spermidine or putrescine was used. Nonradioactive spermine, but not nonradioactive spermidine, competed well with (^{3}H)spermine in binding to the prostate protein. The spermine-binding activity of the cytosol protein could be destroyed by treatment with trypsin or chymotrypsin, but not by pancreatic RNase. The RNase, under the same conditions, eliminated spermine binding to tRNA in the whole cytosol preparation. Millimolar concentrations of MgCl2, ZnCl2, CaCl2, and MnCl2 significantly inhibited the formation of the 3S spermine-protein complex. The spermine-protein interaction was reversible. The ligand could be released from the protein in 0.2 M or higher concentrations of KCl. The formation of the complex was more favorable in Tris-HCl buffer than in the potassium phosphate buffer. With either buffer, there was no change in the binding activity between pH 6.5 and 8.0. The findings raise the possibility that certain cellular actions of polyamines are dependent on specific receptor proteins. (Author Abstract)

489. MECHANISM OF ALKALINE PHOSPHATASE INDUCTION IN CULTURED MAMMARY CARCINOMA CELLS. (PP. 295-301)

Ono T, Koyama H
Control Mechanisms in Cancer. Criss WE, Ono T, Sabine JR, ed. New York, Raven Press, Progress in Cancer Research and Therapy, vol 1, 1976.

The mechanism of alkaline phosphatase induction in cultured mammary carcinoma cells (lines B-6, CHL, FC-1, FM3A, SEN, HT-4, and 7288Ctc-2-1) was investigated. Butyrate, cyclic AMP and 5-bromodeoxyuridine were tested. Butyrate was as effective an inducer of alkaline phosphatase as dibutyryl cyclic AMP (dbcAMP). Cyclic AMP was also effective in inducing the phosphatase although a higher concentration of the natural cyclic nucleotide was necessary to obtain the same effect as dbcAMP. The induction of alkaline phosphatase by butyrate or cyclic AMP was inhibited in the presence of cycloheximide or actinomycin D; this suggests that induction requires continuous synthesis of protein and RNA. The butyrate receptor protein from rat liver is characterized (molecular wt 160,000), its binding to DNA is discussed, and the discovery of a butyrate receptor protein from FM3A cells is announced. It appears that the receptor protein is a positive control factor like receptor proteins for steroid hormones, which bind to the DNA or chromatin and stimulate the transcription of a range of genes. (18 refs)

490. MOUSE TERATOCARCINOMA: ISOLATION AND CHARACTERIZATION OF TWO MUSCLE CELL LINES.

Boon T, Buckingham ME, Dexter DL, Jakob H, Jacob F
Dept. Cell Genet., Coll. France, Paris
Ann Microbiol (Paris); 125(1):13-28 1974

Two clonal cell lines were isolated from "embryoid bodies" (teratoma cells resembling embryos in the blastula stage) from two transplantable mouse teratomas. The PCD2 line, isolated from Stevens OTT-60-50-970 teratoma in 129/Sv mice, resembled skeletal muscle myoblasts. In dense cultures, many cells fused to form myotubes; these contained myofilaments and acetylcholine receptors. Occasionally, some myotubes contracted. Electrophoresis on polyacrylamide gel demonstrated that about 40% of protein synthesized by these cells migrated at the same rate as the principle myosin fraction form striated rat muscle. Cells of the PCD1 line, isolated from Stevens OTT-60-50 in teratoma in 129/Sv mice, never contracted or fused in culture. When they became confluent, they synthesized large amounts of myosin that had the same electrophoretic mobility as cardiac myosin. However, it was not ruled out that this myosin might be smooth muscle myosin. This suggests that PCD1 cells are myocardial in origin. Neither the PCD1 or PCD2 lines have the specific antigen present in multipotential teratocarcinoma cell lines. Both these lines have some H-2 determinants, are aneuploid, and do not induce tumors in isogeneic mice within two months after sc or ip transplantation.

491. NEUROBLASTOMA CULTURE: AN EXPERIMENTAL SYSTEM FOR THE STUDY OF CELLULAR DIFFERENTIATION.

Augusti-Tocco G
Lab. Molecular Embryology, Naples, Italy
Trends Biochem Sci; 1(3):151-154 1976

Mouse neuroblastoma cell lines have been cultured in several laboratories in order to study the molecular events involved in regulating neuron differentiation. Results are summarized that illustrate the possibilities of using this system as a model for differentiation ie, determination of the role of transcriptional control on events such as neurite elongation and RNA and protein content increase. By varying the stability of newly synthesized messenger RNA in the nucleus or by controlling the assembly of the polysomal structure, transcript utilization seems to be selectively controlled in this system. Many markers of neuronal function have been detected. To analyze the correlation between the expression of neuronal-specific functions, clones with low acetylcholinesterase levels have been used. In these cells, a common regulation mechanism exists for the synthesis of acetylcholinesterase and acetylcholine receptors, but choline acetyltransferase appears to be under independent control. The correlation of neurite formation with other specific markers has also been studied. In conclusion, the formation of action potential and the synthesis of neurotransmitters appear to be expressed independently from axon elongation, which may require interaction of developing neurons with their

environment. Membrane modifications that may precede synapse formation have been studied in mixed cultures of neuroblastoma cells and muscle fibers. (18 refs)

492. METABOLISM OF INTRAVENOUSLY INJECTED CADMIUM-BINDING PROTEIN.

Cherian MG, Shaikh ZA

Univ. Western Ontario Medical Center, London, Ontario, Canada

Biochem Biophys Res Commun; 65(3):863-869 1975

The role of metallothionein in the transport of cadmium is explored. Female Sprague-Dawley rats were used as test animals. Synthesis of metallothionein was induced by sc injection of 10 microM CdCl2 /kg at 24 hr intervals for five days. To label the protein with 109Cd, the injection solution contained carrier-free 109Cd, while labeling with 14C or 35S was accomplished by the simultaneous daily ip injection of 20 microCi of radiolabeled cystine solution and CdCl2. Metallothionein from liver tissue was isolated and the protein contents were estimated by atomic absorption and calorimetric procedure. In other experiments, rats were anesthetized, and labeled metallothionein of 109CdCl2 was injected through a cannulated jugular vein at various levels. Bile and urine samples were collected while the rats were under anesthesia. When these rats were sacrificed, liver, kidney, pancreas, and spleen were homogenized, and prepared in 0.25 M sucrose for counting. Kidney supernatant and urine samples were chromatographed on a 0.9 x 60 cm calibrated Sephadex G-75 column. The elution was monitored at 254 nm. 109Cd was counted in a well-type Packard gamma spectrometer equipped with an NaI crystal, and a Packard liquid scintillation spectrometer was used to measure 14C and 35S radioactivity. More than half the injected 109CdCl2 was accumulated in the liver within three hr (103.40 microg). Comparable doses of Metallothionein resulted in a lower liver deposit (32.4 microg). Only a small amount of 109Cd was accumulated in the kidney (3.0 microg) with a 200 micro injected dose. Cadmium in urine excretions after administration of CdCl2 was not significant, although large amounts of 109Cd injected as metallothionein was excreted in the urine. The deposit of 109Cd injected in any form in pancreas and spleen was small. Kidneys took up significant amounts of 109Cd and 35S from injected metallothionein. Fractionation of kidney supernatants showed that about 85% of supernatant 109Cd was bound to metallothionein, however in the kidney supernatant of a rat injected with 35S-metallothionein only 40% of the supernatant radioactivity was recovered. Fractionation of urine samples of rats injected with 109Cd, 14C or 35S labeled metallothionein on Sephadex showed that the major recovered radioactivity was in the metallothionein elution region.

493. INFLUENCE OF PARENTERAL ZINC AND ACTINOMYCIN D ON TISSUE ZINC UPTAKE AND THE SYNTHESIS OF A ZINC-BINDING PROTEIN.

Richards MP, Cousins RJ

Dept. Nutrition, Cook Coll., Rutgers Univ., New Brunswick, NJ 08903

Bioinorg Chem; 4(3):215-224 1975

When Zn65 plus ZnCl2 and C14-cystine are injected into Sprague-Dawley male rats, the liver cytosol (supernate to centrifugation at 42,000 g) chromatographed on G-75 sephadex, and the fractions counted, a peak enriched in both C14 and Zn65 is obtained in the 10-14,000 dalton range. The first peak eluted was a high molecular fraction, the second peak corresponded to elution of small peptides and amino acids. These results indicate that Zn administration (about 10 mg/kg) stimulates the formation of a zinc carrier protein. Similar results are obtainable for rat duodenal mucosae. Injection of actinomycin D 4 hr before Zn abolishes this effect suggesting the need for DNA-transcribed protein synthesis before a response to Zn loading can occur. Effects are furthermore enhanced in Zn-depleted rats. Studies of the clearance of Zn from serum and accumulation in liver support the concept of a specially synthesized zinc-trapping protein in response to zinc loading.

494. EFFECTS OF PLATINUM AND PALLADIUM SALTS ON PARAMETERS OF DRUG METABOLISM IN RAT LIVER.

Holbrook DJ, Washington ME, Leake HB, Brubaker PE

Dept. Biochemistry, Sch. Medicine, Univ. North Carolina, Chapel Hill, NC 27514

J Toxicol Environ Health; 1(6):1067-1079 1976

The effects of various salts of platinum or palladium on the microsomal mixed-function oxidase system of male Sprague-Dawley rats were studied. PtCl4 or Pd(NO3)2 increased the hexobarbital-induced sleeping time in vivo and generally decreased the aminopyrene demethylase and the content of cytochrome P-450 in isolated hepatic microsomes. Dietary administration of platinum or palladium salts for 1 wk generally decreased or had no effect on drug metabolism by isolated microsomes. After 4 or more wk the salts had no effect on the parameters. The inability of long-term dietary salts to decrease the parameters of drug metabolism may be due to the synthesis of a $Pt^{4}+$ or $Pd^{2}+$ binding protein that sequesters the intracellular cations. The addition of 0.15 to 0.2 milliM PtCl4 or 0.2 to 0.3 milliM Pd(NO3)2 inhibited the aminopyrine demethylase of isolated hepatic microsomes by 50%. Although levels of platinum in tissues of experimental animals can attain levels that are nominally sufficient to inhibit the hepatic enzymes, the amounts that must be administered to animals far exceed an anticipated environmental exposure. (16 Refs)

495. IMMUNE PRECIPITATION OF ^{238}PU(IV)-TRANSFERRIN (MEETING ABSTRACT).

Durbin PW, Heavy LR, Garcia JF

Div. Biology and Medicine, Lawrence Berkeley Lab., Berkeley, CA 94720

Radiat Res ; 67(3):578-579 1976

A double antibody immune method conclusively identified transferrin (TF) as the main Pu-binding protein in plasma and demonstrates that neither Fe(III) nor Pu(IV) interferes with the binding of TF to antibody. Antibody to human TF (HTF) was raised in rabbits. The soluble HTF-antibody complex was precipitated by a second reaction with serum from goats immunized against rabbit gamma-globulin and was collected quantitatively by centrifugation. The rabbit and goat antisera and their globulin fractions (separated on Sephadex gel or by precipitation with 33% saturated (NH4)2SO4) were titered using ^{125}I HTF or ^{59}Fe-HTF. ^{238}P-lutonium (IV) was incubated with HTF or human plasma. Filtration on Sephadex gel showed that about 95% of both ^{59}Fe and ^{238}Pu were bound to protein after incubation with either HTF or plasma. The immune precipitate of ^{59}Fe-HTF contained about 95% of the ^{59}Fe whether the reactants were antisera or their globulin fractions. However, the amount of ^{238}Pu in the immune precipitate of ^{238}Pu-HTF depended on the purity of the reactants; antisera, 30 to 50%, globulins separated by (NH4)2SO4, 60 to 70%; globulins separated on Sephadex gel, 80 to 85%. The amount of ^{238}Pu in the immune precipitate of HTF from ^{238}Pu-labeled plasma was only slightly less than when the antigen was pure ^{238}Pu-HTF. The complexing species present in whole antisera and in lesser amounts in crude globulin fractions apparently promoted partial dissociation of Pu-HTF.

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